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Precipitation, snowpack, stream-water ion chemistry, and flux in a northern Michigan watershed, 1982–1991

Robert Stottlemyer and David Toczydlowski

Abstract: Input–output budgets, snowpack ion content and loss, and change in stream-water chemistry during snowmelt were quantified for 10 years in a small watershed in upper Michigan. Annual precipitation (centimetres) and H^+ and SO_4^{2-} inputs declined during the decade. Precipitation SO_4^{2-} concentration decreased, with two thirds of the decline occurring in winter. Winter precipitation and H^+, NH_4^+, NO_3^-, and SO_4^{2-} inputs declined during the decade. Reduced precipitation SO_4^{2-} concentration accounted for a third of the reduction in winter SO_4^{2-} input. Up to snowpack peak water equivalent, snowmelt totalled one third and ion loss totalled 37–58% of cumulative precipitation inputs. Midwinter snowmelt was attributed to unfrozen soils and intermittent thaws. About 90% of snowmelt entered unfrozen soil, where soil processes significantly altered its chemistry. The watershed retained >99% of snowmelt H^+, >95% of NH_4^+, and >80% of NO_3^-. At peak snowmelt, stream-water acid neutralization capacity declined by 360 $\mu equiv.\cdot L^{-1}$, with >90% resulting from dilution. Stream-water NO_3^- concentration declined >85% during snowmelt and SO_4^{2-} concentration changed <10%. Soil SO_4^{2-} desorption was probably the major process buffering change in stream-water SO_4^{2-} concentration.

Résumé : Les bilans d'entrée–sortie, la concentration et la déperdition d'ions de la couche de neige et les changements de la chimie de l'eau pendant la fonte des neiges ont été quantifiés pendant 10 ans dans un petit bassin-versant du haut Michigan. Les précipitations annuelles (centimètres) et les apports de H^+ et de SO_4^{2-} ont baissé au cours de la décennie. La concentration de SO_4^{2-} dans les précipitations a baissé, les deux tiers de la baisse se produisant en hiver. Les précipitations hivernales et les apports de H^+, de NH_4^+, de NO_3^- et de SO_4^{2-} ont baissé au cours de la décennie. La baisse de la concentration de SO_4^{2-} dans les précipitations correspondait à un tiers de la réduction de l'apport hivernal de SO_4^{2-}. Jusqu'au moment du maximum de l'équivalent en eau de la couche de neige, la fonte de la neige a totalisé un tiers et la déperdition d'ions 37–58% des apports cumulatifs des précipitations. La fonte des neiges du milieu de l'hiver a été attribuée à la présence de sols non gelés et à des dégels intermittents. Environ 90% de l'eau de fonte pénétrait dans le sol non gelé où les processus édaphiques modifiaient notablement sa chimie. Le bassin-versant retenait >99% du H^+, >95% du NH_4^+ et >80% du NO_3^- de l'eau de fonte. Au maximum de la fonte des neiges, la capacité de neutralisation de l'acide de l'eau des rivières a baissé de 360 $\mu equiv.\cdot L^{-1}$, dont >90% provenait de la dilution. La concentration de NO_3^- dans l'eau des rivières a baissé de >85% pendant la fonte des neiges, tandis que la concentration de SO_4^{2-} changeait de <10%. C'est probablement la désorption du SO_4^{2-} du sol qui constituait le principal processus faisant tampon contre les changements de la concentration du SO_4^{2-} dans l'eau des rivières.
[Traduit par la Rédaction]

Introduction

In the glaciated Upper Great Lakes Basin, moderate to high inputs of acidic deposition occur. Annual H^+ input averages 195 $equiv.\cdot ha^{-1}$, NH_4^+, 190 $equiv.\cdot ha^{-1}$, NO_3^-, 225 $equiv.\cdot ha^{-1}$, and SO_4^{2-}, 300 $equiv.\cdot ha^{-1}$ (National Atmospheric Deposition Program 1982–1991). Along the south shore of Lake Superior, snowfall can equal half of the annual precipitation (Eichenlaub et al. 1990; Stottlemyer and Rutkowski 1990), and the snowpack may temporarily store up to 50% of annual precipitation (Stottlemyer 1987).

In this region, half of the annual stream-water runoff occurs during and immediately after snowmelt (Nicholson 1988; Stottlemyer and Toczydlowski 1991). Snowmelt can cause sharp changes in stream-water chemistry by dilution, which increases the chance of acidification from high ion concentrations in initial snowmelt (Johannessen and Henriksen 1978). Sensitive aquatic ecosystems are especially at risk (Hagen and Langeland 1973). In the Upper Great Lakes region, such systems occur where substrates are dominated by Precambrian granites and glacial outwash plains (Jeffries et al. 1979; Kelso et al. 1986; Rascher et al. 1987). Anthropogenic acidic deposition has been implicated in recent lake acidification in central Ontario (LaZerte and Dillon 1984). However, much of the region has well-buffered soils and surface water, especially in streams (Overton et al. 1986; Stottlemyer and Toczydlowski 1991; Wiener and Eilers 1987). Such streams usually respond little to snowmelt H^+ inputs but can be altered by snowmelt dilution and inputs of NO_3^- and SO_4^{2-}.

The relationships between precipitation, snowpack, snowmelt, and stream-water chemistry are complex and subject to much temporal and spatial variability (Jeffries 1990). Winter precipitation chemistry concentrations often differ from those during the snowfree season. The chemistry of rain during late winter can be very different from snow (Topol 1986). A snowpack

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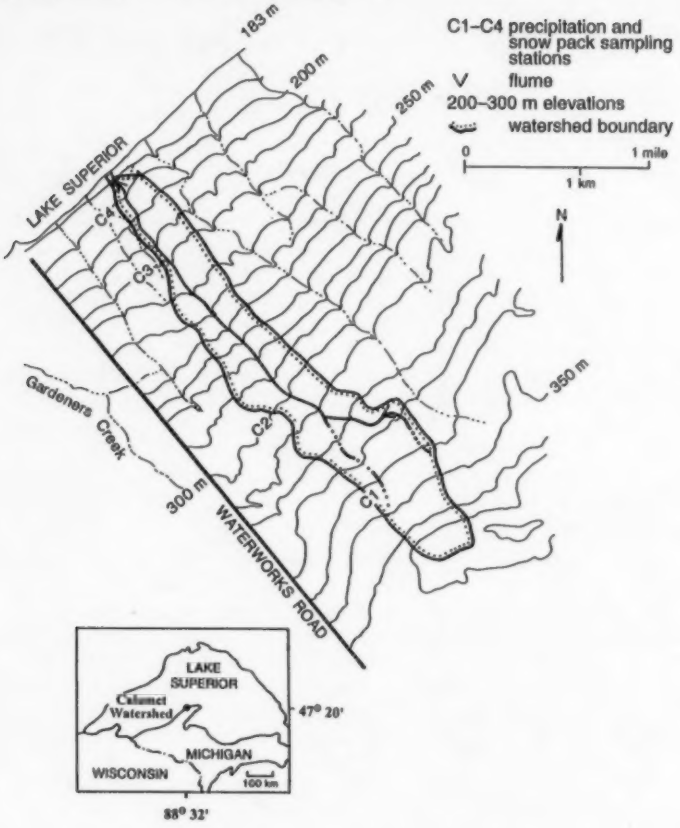
Fig. 1. Calumet watershed (stippled boundary), Keweenaw Peninsula, Michigan.



integrates inputs of wet (rain and snow) and dry deposition (Dewalle 1987; Jones 1987), but freeze–thaw cycles and rain rapidly redistribute snowpack solutes, which alters their time of release and concentration (Barry and Price 1987; Jones 1987). Snowpack chemistry is further changed by the dissolution of particulates and leaching of organic debris within it, especially later in winter (Dewalle 1987; Hornbeck and Likens 1974; Jones and Sochanska 1985). The presence of frozen soils is a major factor affecting the timing and quality of snowmelt. Frozen soils beneath the snowpack delay snowmelt release, isolate snowmelt solutes from interacting with the forest floor, and increase the amount of snowmelt solutes directly reaching a stream (Pierson and Taylor 1985). When soils are unfrozen throughout winter, the premelt chemical stability of the snowpack is affected by convective heat flux. Unfrozen soils permit snowmelt to enter the soil, where its chemistry is rapidly altered, even in poorly developed soils or talus (Caine and Thurman 1990; Campbell et al. 1995; Hazlett et al. 1992).

In the Calumet watershed, located along the south shore of Lake Superior (Fig. 1), annual snowfall is >500 cm and the peak snowpack water equivalent (PWE) can exceed 30 cm. Where large snowpacks occur in this region, soils remain unfrozen (Hazlett et al. 1992; Rascher et al. 1987; Stottlemyer and Rutkowski 1990). At Calumet, unfrozen soils coupled with midwinter thaws can result in >50% of the snowpack solute load and >10% of total snowmelt being released before peak snowmelt. Throughout winter, <10% of total snowmelt occurs as overland flow (Stottlemyer and Toczydlowski 1991). During winter and spring, these conditions affect ecosystem processes, such as nutrient availability from snowmelt (Fahey 1978; Rascher et al. 1987; Semkin and Jeffries 1988), soil mineralization, nitrification, microbial immobilization, and regulation of soil moisture content (Kane et al. 1991). These processes can, in turn, alter the chemistry of snowmelt as it passes through the soil before reaching the aquatic ecosystem (Foster et al. 1989; Hazlett et al. 1992; Stottlemyer and Toczydlowski 1996). The steady release of solutes in snowmelt throughout winter and passage of snowmelt through the forest floor and mineral soils can reduce the potential impacts of snowpack contaminants on aquatic ecosystems during peak runoff.

Although much is known about the relationship of snowmelt to changes in surface-water chemistry in some locations in the region (LaZerte and Dillon 1984; Tranter et al. 1987), few studies have been conducted linking change in precipitation and snowpack chemistry to stream-water chemistry in areas of high snowfall during a multiyear period to permit development of more general hypotheses. The objectives of this paper are (*i*) to summarize 10 years of mass balance data that examine trends, both among and within winters, in patterns

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of precipitation inputs, snowpack ion content, and snowmelt and (*ii*) to relate these processes to seasonal variation in stream-water chemistry for selected ions (Ca^{2+}, H^+, NH_4^+, NO_3^-, and SO_4^{2-}).

Site description

The Calumet watershed (176 ha) is a first-order catchment vegetated by 65-year-old sugar maple (*Acer saccharum*) and white birch (*Betula papyrifera*) (Fig. 1). The watershed is adjacent to Lake Superior and has a northwest aspect, uniform slope, and moderate topographic relief. The bedrock is Precambrian metamorphosed Portage Lake volcanics overlain with alkaline glacial till and old beach deposits. Soils are primarily typic haplorthods. An almost impervious soil layer (ortstein) occurs at a 1.5- to 2-m depth. Weathering of the alkaline till results in moderately buffered stream water (Stottlemyer and Toczydlowski 1991). Calcium and HCO_3^- are the dominant ions (equivalent basis) in stream water, and H^+ and SO_4^{2-} are the dominant ions in precipitation. A National Atmospheric Deposition Program (NADP) site, 21 km from the watershed, has been in operation since 1982. The National Oceanographic and Atmospheric Administration (NOAA) operates a primary weather station at the Houghton County airport 6 km from station C1 at the same elevation.

The climate on the south shore is substantially modified by Lake Superior, which rarely freezes over. The open water moderates winter temperature extremes and contributes to the region's high (>500 cm·yr^{-1}) snowfall. These conditions, coupled with daily maximum air temperatures approaching 0°C (Stottlemyer and Rutkowski 1990) and unfrozen soils, result in periodic midwinter thaws and small, but steady, snowmelt throughout much of winter, a pattern quite different from those seen at sites farther away from Lake Superior's high snowfall zone (Cadle et al. 1984; Eichenlaub et al. 1990; Kelso et al. 1986; Semkin and Jeffries 1988).

Methods

In 1979, four precipitation and snowpack monitoring stations were located at intervals of increasing elevation in the watershed (C4 (190 m, near the elevation of Lake Superior), C3 (240 m), C2 (295 m), and C1 (350 m)) (Fig. 1). Each station was equipped with a bulk collector (36-cm diameter) to measure both precipitation quality and quantity. We did not shield these collectors primarily because our comparison sites, NADP stations MI99 and MI97, are not shielded. Early studies by the NADP were not conclusive in demonstrating a significant effect on precipitation amount from shielding (Simmons and Bigelow 1990). A snow survey transect (five sampling points) was set up at each station where snow water equivalent (SWE) and depth were determined using a Mount Rose snow sampler. At each station, a snow sample was collected for chemical analyses using a 5 cm diameter PVC tube.

Stream-water discharge was continuously measured year-round using a 30 cm wide Parshall flume equipped with a Stevens F recorder (1979–1990) or a Stevens pressure transducer and LiCor model 1000 datalogger (1990–1991). Ice blockage is not a problem and the flume is unheated.

Precipitation, snowpack, and stream samples were collected weekly for chemical analyses. During snowmelt, stream samples were collected daily at both maximum and minimum discharge (Stottlemyer and Toczydlowski 1991). Stream samples were collected above the flume using brown polyethylene 500-mL bottles. Bottles were washed for re-use, rinsed three times in DI water, and rinsed with stream water twice before retaining a sample. During the snow-free season, precipitation amount does not change with elevation (R. Stottlemyer, unpublished data), and precipitation was sampled weekly at station C1, using a plastic Taylor rain gauge equipped with a qualitative prerinsed ashless filter to minimize evaporation and dust. The rain gauge was changed weekly, and filters and the rain-gauge tube were washed in DI water before use. Stream water was sampled on the same day.

Samples were immediately transported to the laboratory at Michigan Technological University. Snow samples were melted at room temperature in prerinsed, covered polyethylene containers. pH, specific conductance, and alkalinity (titration with 0.02 M H_2SO_4 to pH 4.5) were determined when samples reached room temperature (<24 h). Filtered (prerinsed 0.45-μm filters) subsamples were refrigerated (2°C) for up to 3 weeks and chemical analyses for Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+, Cl^-, NO_3^-, PO_4^-, and SO_4^{2-} were conducted using ion chromatography (Dionex model 2020, see Johnson and Haak 1983).

Weekly snowpack moisture loss was estimated at each station for week t by summing $t-1$ SWE plus the precipitation for week t minus the SWE for week t. The change in snowpack ion content and ion loss in snowmelt were estimated for each ion with the same approach as for determining change in snowpack moisture.

Trends during the decade in annual precipitation inputs and stream-water outputs for selected variables are presented. In all cases, results are for the water year, which for Calumet is October 1 – September 30. In addition, trends through the decade for winter-season means and sums for selected variables are also presented. To analyze within-winter trends in precipitation, snowpack, and stream water, the primary focus of this paper, each winter period was divided into elapsed weeks from the start of permanent snowpack. Regardless of what date the snowpack became established, week 1 was the 1st week of permanent snowpack, and so on. The permanent snowpack averaged 18 weeks in duration. Change in weekly SWE and ion content were estimated by comparing the mean from 1 week ($n = 10$ for the decade) with those from another. The precipitation record presented was generally 21 weeks, which covered the maximum length of permanent snowpack.

Data were analyzed and graphics prepared using SYSTAT subroutines (Wilkinson 1990). When comparing mean annual, winter, or weekly trends within winter, SYSTAT Multivariate General Linear Hypothesis (MGLH) subroutines were used. MGLH presents r^2, the standard error of the estimate, and analysis of variance (ANOVA) tables for estimating fit of the model. For annual and winter trends, the number of observations was usually 10. All annual values were for the water year. For weekly trends, the number of weeks varied from about 13 for snowmelt to 25 for stream water.

Results

Precipitation

Annual precipitation averaged 80 ± 20 cm (range 54–109 cm) and declined ($p < 0.01$, $r^2 = 0.61$, $n = 10$) on average 5 cm·yr^{-1} throughout the decade (Table 1, Fig. 2). Annual precipitation was about 15% less and winter precipitation (December 1 – April 23) was 30% greater (Eichenlaub et al. 1990; National Atmospheric Deposition Program 1982–1991) than in other portions of the region.

Winter precipitation averaged 34 ± 11% of the annual total and declined ($p < 0.01$, $r^2 = 0.68$) on average 2.6 cm·yr^{-1} during the decade (Table 2, Fig. 2) or half of the annual decline. Winter precipitation was greater at the highest elevation station (C1, Fig. 3). Precipitation totals increased with elevation when winds were off the lake. Little difference in precipitation amount with elevation occurred when storms were from the

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Table 1. Time trends in annual precipitation input and stream-water output for selected ions, 1982–1991, Calumet watershed.

	Equation
Precipitation	
Amount	cm = 105 – 5.2(yr)
Ca^{2+}	equiv.·ha^{-1} = 156 – 7.8(yr)
H^+	equiv.·ha^{-1} = 560 – 39(yr)
NH_4^+	equiv.·ha^{-1} = 170 + 1.5(yr)
NO_3^-	equiv.·ha^{-1} = 283 – 6.3(yr)
SO_4^{2-}	equiv.·ha^{-1} = 545 – 26(yr)
Stream water	
Amount	cm = 41 – 1.3(yr)
Ca^{2+}	equiv.·ha^{-1} = 3550 – 100(yr)
H^+	equiv.·ha^{-1} = 5 – 0.3(yr)
NH_4^+	equiv.·ha^{-1} = 7 – 0.3(yr)
NO_3^-	equiv.·ha^{-1} = 24 + 2(yr)
SO_4^{2-}	equiv.·ha^{-1} = 620 – 15(yr)

Note: In the regression equation, the constant is the *y*-intercept when year is zero. The second number is the slope of the line or the number of units of change in *y* with a change as 1 year.

Table 2. Time trends in winter precipitation input, peak snowpack ion content, and stream-water runoff and ion output during the decade (1982–1991).

	Equation
Winter precipitation	
Amount	cm = 47 – 2.6(yr)
H^+	equiv.·ha^{-1} = 100 – 6(yr)
NH_4^+	equiv.·ha^{-1} = 64 – 4(yr)
NO_3^-	equiv.·ha^{-1} = 135 – 9(yr)
SO_4^{2-}	equiv.·ha^{-1} = 170 – 15(yr)
Peak snowpack	
Amount	cm = 24 – 0.2(yr)
H^+	equiv.·ha^{-1} = 135 + 10(yr)
NH_4^+	equiv.·ha^{-1} = 8 + 2(yr)
NO_3^-	equiv.·ha^{-1} = 62 – 2(yr)
SO_4^{2-}	equiv.·ha^{-1} = 82 – 5(yr)
Stream water	
Amount	cm = 33 – 0.1(yr)
Ca^{2+}	equiv.·ha^{-1} = 1750 – 55(yr)
H^+	equiv.·ha^{-1} = 0.5 – 0.02(yr)
NH_4^+	equiv.·ha^{-1} = 2 + 0.3(yr)
NO_3^-	equiv.·ha^{-1} = 23 + 0.8(yr)
SO_4^{2-}	equiv.·ha^{-1} = 370 – 0.3(yr)

Fig. 2. Annual (water year) precipitation (—), stream-water runoff (– –), total winter precipitation (- - -), and mean snowpack peak water equivalent (PWE; ·····), 1982–1991, Calumet watershed, Michigan.

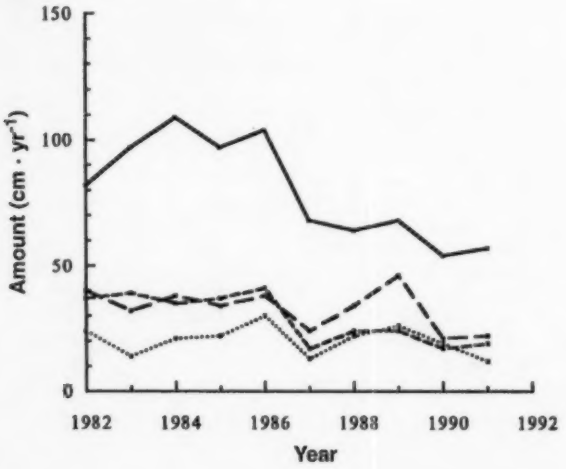
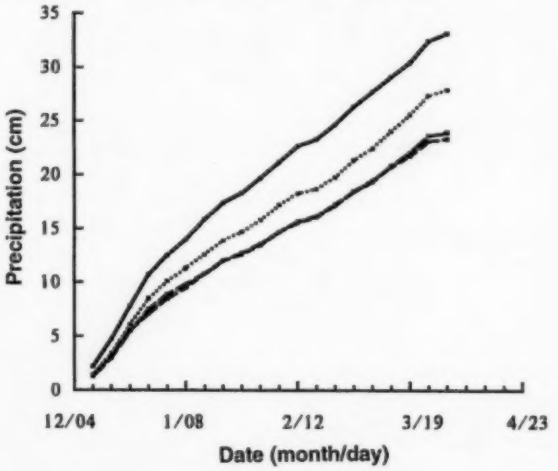


Fig. 3. Cumulative winter precipitation with elevation, 1982–1991, Calumet watershed, Michigan: station C1, 350 m elevation (—); C2, 295 m (– –); C3, 240 m (- - -); and C4, 190 m (·····).



direction of S or SW. A switch in prevailing wind direction occurred by mid-March (Stottlemyer and Toczydlowski 1991). Within winter, average weekly precipitation declined 0.1 cm ($p < 0.05$, $r^2 = 0.27$; Table 3). The standard deviation was 76% of mean weekly precipitation amount.

Except for NH_4^+, annual precipitation ion input declined during the study (Table 1, Fig. 4). The declines were significant for H^+ ($p < 0.001$, $r^2 = 0.84$) and SO_4^{2-} ($p < 0.05$, $r^2 = 0.51$). The decline in ion input was due primarily to the decrease in precipitation amount. Sulfate exhibited the only significant decline in mean annual precipitation concentration (1.5 μequiv.·L^{-1}·yr^{-1}, $p < 0.05$, $r^2 = 0.62$) during the study. About 70% of the SO_4^{2-} concentration decline occurred in winter. Precipitation ion concentration did not change with elevation.

Total winter precipitation SO_4^{2-} input declined more rapidly than total precipitation amount (Table 2, Fig. 5). For other ions, the winter decrease was more similar to the decline in precipitation amount. During the study, total winter precipitation amount declined 55%, and H^+, NH_4^+, NO_3^-, and SO_4^{2-} declined 60, 63, 67, and 88%, respectively. All trends were significant ($p < 0.05$, $r^2 > 0.48$).

Within winter, only H^+ input showed a significant decline ($p < 0.05$, $r^2 = 0.39$) in weekly input (Table 3). Weekly precipitation input of most ions except NO_3^- increased by mid-March

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Table 3. Within-winter trends in average weekly precipitation inputs, snowpack content, and output in stream water up to snowpack peak water equivalent (PWE).

	Equation
Precipitation	
Amount	cm = 2.2 – 0.1(week)
Ca^{2+}	equiv.·ha^{-1} = 2 + 0.01(week)
H^+	equiv.·ha^{-1} = 7 – 0.3(week)
NH_4^+	equiv.·ha^{-1} = 2 – 0.05(week)
NO_3^-	equiv.·ha^{-1} = 5 – 0.2(week)
SO_4^{2-}	equiv.·ha^{-1} = 4 – 0.1(week)
Snowpack	
Amount	cm = 4 + 1(week)
Ca^{2+}	equiv.·ha^{-1} = 0 + 3(week)
H^+	equiv.·ha^{-1} = 11 + 3(week)
NH_4^+	equiv.·ha^{-1} = 4 + 1.5(week)
NO_3^-	equiv.·ha^{-1} = 7 + 3(week)
SO_4^{2-}	equiv.·ha^{-1} = 7 + 2(week)
Stream water	
Amount	cm = 0.03 – 0.001(week)
Ca^{2+}	equiv.·ha^{-1} = 22 – 0.3(week)
H^+	equiv.·ha^{-1} = 0.002 – 0.0001(week)
NH_4^+	equiv.·ha^{-1} = 0.02 + 0.001(week)
NO_3^-	equiv.·ha^{-1} = 0.25 + 0.01(week)
SO_4^{2-}	equiv.·ha^{-1} = 5 – 0.1(week)

(Fig. 6). During the last 4 weeks of snowpack, mean weekly precipitation declined 23% relative to the average weekly amount before snowpack PWE (Fig. 6*a*), but weekly Ca^{2+} deposition, H^+, NH_4^+, and SO_4^{2-} increased 83, 33, 92, and 78%, respectively, and NO_3^- declined 15% (Figs. 6*b*–6*f*).

Snowpack and snowmelt

The mean snowpack PWE was 21 ± 5 cm (Fig. 2). No significant correlation existed between total winter precipitation, mean watershed SWE, and SWE at any individual station. A permanent snowpack formed in the 1st week in December on average and remained until early in the 1st week of April (Fig. 6*a*). Snowpack PWE occurred about the 1st week of March, and variation (standard deviation) was 31% of the mean (Fig. 7). Variation in weekly SWE came primarily from differences in mean SWE among winters (Fig. 8*a*). Variation in weekly SWE among years averaged 41% of the mean (Figs. 6*a* and 8*a*).

Throughout winter, SWE increased with elevation (Fig. 7). Up to snowpack PWE, the mean SWE increased ($p < 0.001$, $r^2 = 0.92$) 1 cm·$week^{-1}$ (Table 3, Fig. 6*a*), and cumulative precipitation was correlated to weekly SWE (Pearson, $p < 0.001$) and cumulative snowmelt ($p < 0.05$). Some SWE loss occurred throughout winter (Fig. 6*a*). At snowpack PWE, the SWE was 7 cm less than cumulative precipitation, which almost equalled estimated snowmelt (8 cm) to that date. Up to snowpack PWE, >50% of the cumulative snowmelt occurred during the 1st week of permanent snowpack. The first major midwinter thaw occurred about 6 weeks after permanent snowpack formation, and the final snowmelt period averaged 6 weeks.

The only significant trend in snowpack PWE or ion content was the increase in NH_4^+ ($p < 0.05$, $r^2 = 0.54$) (Table 2). Up to snowpack PWE, the weekly increase in snowpack content of all ions was significant ($p < 0.01$, $r^2 > 0.77$). For all ions except NH_4^+, the increase in snowpack content was more rapid than SWE (Table 3, Figs. 6*a*–6*f*.). At snowpack PWE and the start of final snowmelt, the snowpack content of Ca^{2+} was 81% of cumulative precipitation inputs and H^+, NH_4^+, NO_3^-, and SO_4^{2-} were 60, 45, 69, and 73%, respectively (Figs. 6*b*–6*f*).

Up to snowpack PWE, there was a positive correlation between cumulative precipitation ion inputs and snowpack content ($p < 0.01$) and, except for NO_3^-, cumulative precipitation ion inputs and cumulative snowmelt ion output ($p < 0.05$). By snowpack PWE, the estimated total snowmelt Ca^{2+} loss was 37% of cumulative precipitation inputs and H^+, NH_4^+, NO_3^-, and SO_4^{2-} were 46, 58, 37, and 45%, respectively.

Fig. 4. Annual precipitation input of selected ions, 1982–1991, Calumet watershed, Michigan: hydrogen (- - -), ammonium (– –), nitrate (---), sulfate (·····), annual precipitation (—).

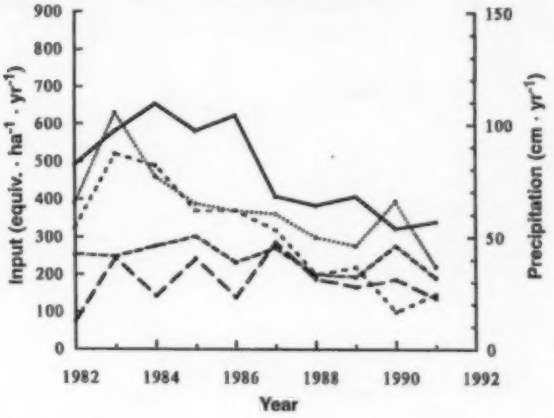
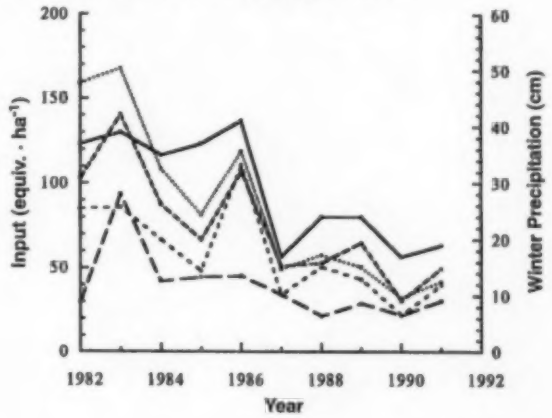


Fig. 5. Time trend in winter precipitation input of selected ions, Calumet watershed, Michigan: hydrogen (- - -), ammonium (– –), nitrate (---), sulfate (·····), winter precipitation (—).



Stream-water output

Annual stream-water runoff (34.5 cm, range 21–46 cm) for the study period was 43 ± 15% of precipitation total (range 54–109 cm) (Fig. 2). Peak stream-water runoff occurred the

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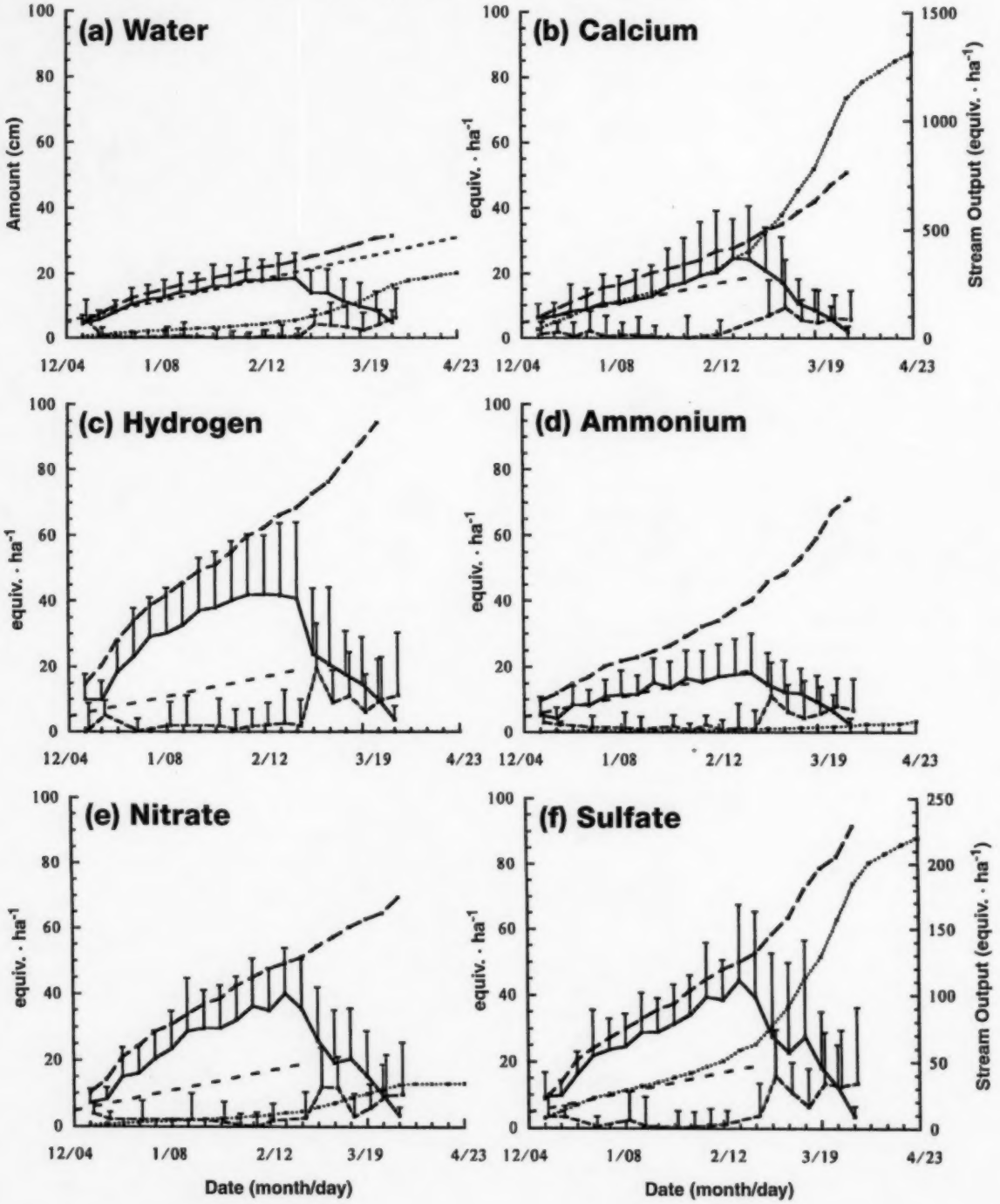
Fig. 6. Mean cumulative weekly inputs and outputs to the Calumet watershed, Michigan, 1982–1991: (*a*) precipitation amount (– –), snowpack water equivalent (SWE; —), snowmelt (---), cumulative stream-water output (····), and regression line of SWE (- - -) up to snowpack peak water equivalent (week of Feb. 26); (*b–f*) various ions as precipitation input (– –), snowpack content (—), snowmelt input (---), cumulative streamwater output (····), and slope of regression for SWE (- - -) from *a*, 1982–1991, Calumet watershed, Michigan. Error bars are slightly offset to avoid overlap. Cumulative stream-water H^+ output (*c*) was so low that it did not rise above the *x* axis.



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week of April 2 (Fig. 9*a*). Annual stream-water runoff declined ($p < 0.05$, $r^2 = 0.77$) about 1 cm·yr^{-1} (Table 1, Fig. 2).

About 56% of annual stream-water runoff, or 20 cm, occurred during winter. Over 30% of this total occurred in April. A positive relation ($p < 0.01$, $r^2 = 0.64$) existed between winter and annual stream-water runoff from the watershed and winter runoff and date of peak stream-water discharge ($p < 0.05$, $r^2 = 0.48$). The later the date of peak stream-water discharge, the greater its amount.

With the exception of NO_3^-, annual stream-water ion output declined during the study, but there were no significant trends (Table 1). The ratio of annual precipitation ion input to watershed output showed no significant trends except for a decrease in NO_3^- ($p < 0.05$, $r^2 = 0.43$).

Average winter precipitation ion input – stream-water output ratios were correlated ($p < 0.01$) only for NH_4^+. Mean winter stream-water NH_4^+ output increased ($p < 0.05$, $r^2 = 0.47$) during the study (Table 2).

Up to snowpack PWE, within-winter weekly stream-water output of all ion species except NH_4^+ and NO_3^- declined gradually (Table 3); however, the changes were minor and there were no significant trends. Stream-water ion output responded little to midwinter snow-melt inputs (Figs. 6*a*–6*f*). At peak snowmelt in early March, mean weekly stream-water runoff was about 700% (Fig. 9, Table 4) of the mean of weekly runoff before March, and ion output was proportional to the increase in stream-water runoff. During the 20-week snowpack period, the cumulative watershed runoff was 53 ± 20% of total precipitation; Ca^{2+} output was 2100 ± 1450% of measured input; H^+, 0.2 ± 0.1%; NH_4^+, 3 ± 6%; NO_3^-, 18 ± 7%; and SO_4^{2-}, 200 ± 80% (Figs. 6*a*–6*f*).

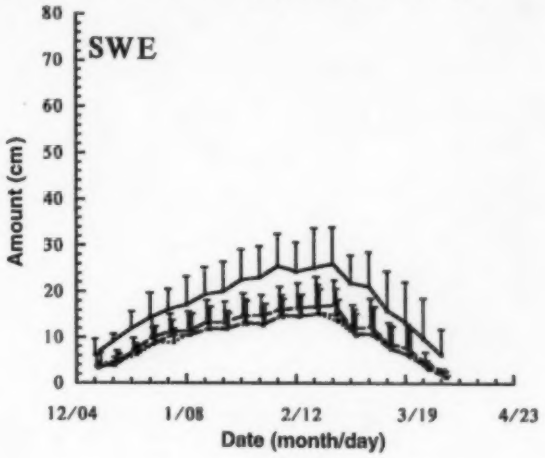
Dilution and change in watershed flow paths during snowmelt brought marked changes in stream-water ion concentration (Fig. 9). Stream-water ion concentrations increased at the initiation of the final snowmelt period (Figs. 6*a*, 9*b*, and 9*c*). The decline in acid neutralization capacity (ANC) from midwinter to peak stream-water discharge averaged 360 μequiv.·L^{-1}. Dilution accounted for 94% of the decline. Stream-water H^+ was not plotted because its average peak concentration was <1 μequiv.·L^{-1}. No trend was evident in stream-water NH_4^+ concentration throughout winter. Nitrate concentrations were highly variable from year to year, especially near the onset of peak snowmelt. Stream-water SO_4^{2-} concentrations were an order of magnitude greater than NO_3^-. Sulfate concentration changed <10% during snowmelt and then gradually declined relative to premelt concentrations.

Discussion

Precipitation

The significant decline in total annual precipitation during the study was within the range of the previous 30 years (Fig. 10). The decrease since the mid-1980s occurred throughout the region (Jones et al. 1993; National Oceanic and Atmospheric Adminstration, Houghton County Airport, MI; Schindler et al. 1990). The precipitation trend shows the importance of natural variation as a major factor in determining atmospheric contaminant input in this region (Jeffries et al. 1988) and the need for longer term studies to assess the effects of such inputs (Likens and Bormann 1995). Variation in annual precipitation

Fig. 7. Change in average snowpack water equivalent (SWE) and standard deviation with elevation, 1982–1991, Calumet watershed, Michigan. Error bars are slightly offset to avoid overlap. Elevations: 350 m (—), 295 m (– –), 240 m (---), 190 m (····).



during the study, 25%, was higher than expected, 16%, for sites with similar annual precipitation amounts (Conrad 1941). The watershed location within the snow belt along the south shore of Lake Superior likely accounts for this.

Within winter, there was no trend in weekly precipitation variation (standard deviation) as a percentage of the mean. Differences among years in weekly precipitation accounted for most of the variation. Variation in mean precipitation with elevation averaged 17% (Fig. 3). We cannot readily explain the large increase in precipitation and SWE (Fig. 7) at station C1. Interaction between elevation and distance from the lake could be a factor.

Few reliable precipitation chemistry data exist for any U.S. region except for New England before 1975 (Gibson 1986). Precipitation chemistry data collected from 1970 to 1982 at the Experimental Lakes Area in northwestern Ontario showed no trend in ion concentration up through 1981 (Linsey et al. 1987). At Calumet, the significant decline in annual precipitation input of most ions is consistent with findings for 1980–1992 from the NADP network (Lynch et al. 1995). Their study showed significant declines in volume-weighted concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+, SO_4^{2-}, and Cl^- in this region. Only the decreases in NH_4^+ and NO_3^- concentrations were not significant. The use of bulk collectors at Calumet could increase variation in precipitation chemistry, especially for base cations (C_B), because of local dust inputs (Glass and Loucks 1986). This may account for the lack of C_B concentration trends in this study.

The reduced annual ion input during the decade was mainly due to decreased precipitation amount and not to change in ion concentration (Table 1, Fig. 4). The significant decline in precipitation SO_4^{2-} concentration was the exception and could reflect reduced regional and national sulfur emissions (Husar 1986; Likens 1992; National Acid Precipitation Assessment Program 1990). The decline in sulfur emissions for this region began in the early 1970s, before precipitation chemistry was routinely monitored. From 1970 to 1985, sulfur emissions in

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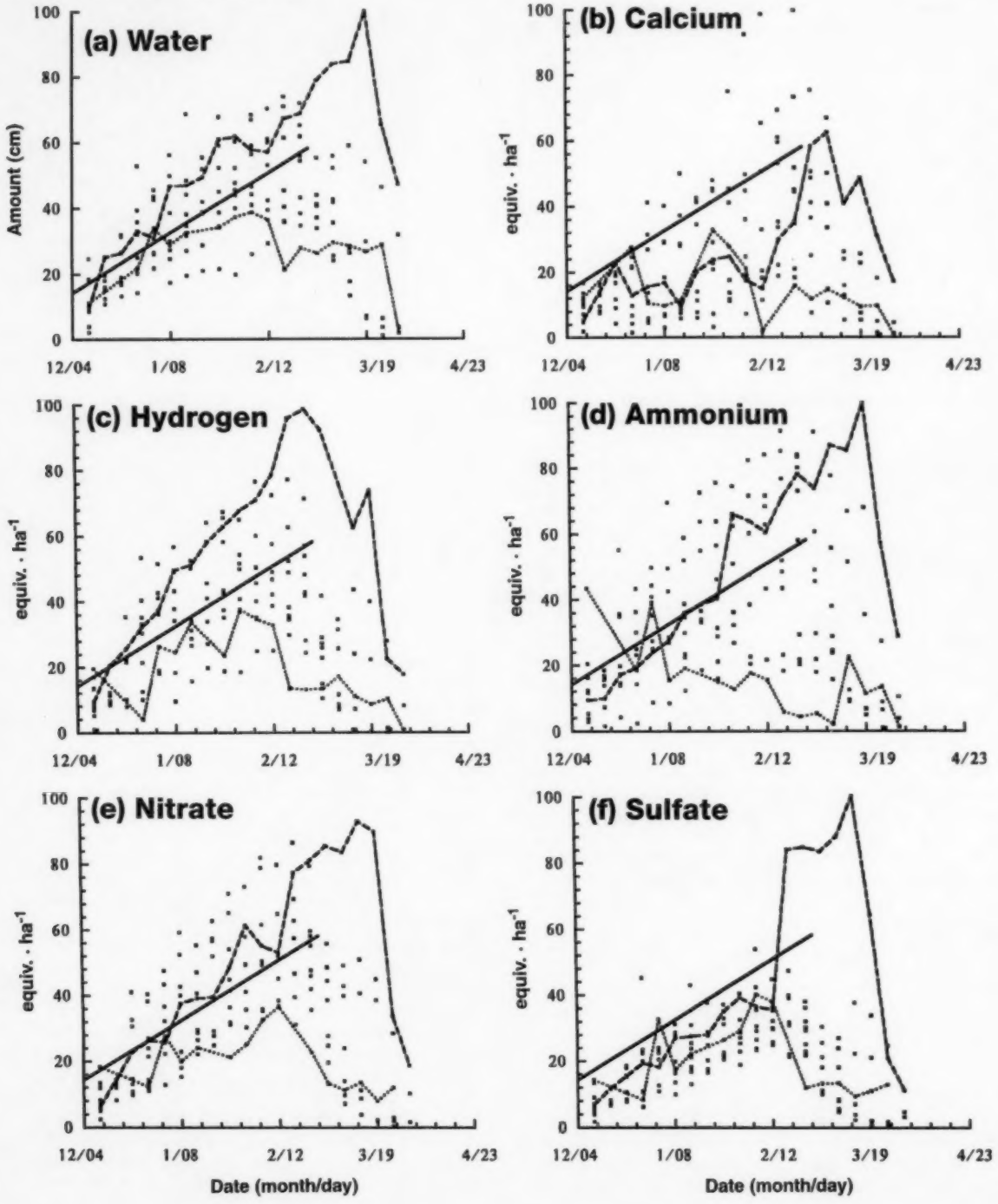
Fig. 8. Scatter plots of mean weekly snowpack variables in the Calumet watershed, Michigan. (*a*) SWE and slope of regression for SWE up to snowpack peak water equivalent (PWE). Lines connect mean weekly SWE for 1986 (---), the year of maximum mean SWE during the study, and mean weekly SWE for 1983 (····), which was the year of minimum mean SWE. (*b–f*) Various ions as snowpack content and slope of regression for SWE up to peak snowpack water equivalent (Feb. 26). Lines connect weekly snowpack contents for 1986 (---), maximum mean SWE, and 1983 (····), minimum mean SWE.



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the eastern U.S. declined an average of 3%·yr^{-1}. At Calumet, precipitation SO_4^{2-} concentration also declined 3%·yr^{-1} after 1982. This agreement between emissions and precipitation chemistry could partly be by chance. For a given area, sulfur emissions and SO_4^{2-} deposition are generally not correlated (National Acid Precipitation Assessment Program 1990). The decrease in annual SO_4^{2-} input during the decade was large (260 equiv.·ha^{-1}) relative to the range of deposition rates near the Lake Superior Basin. Annual SO_4^{2-} deposition west of Lake Superior is about 200 equiv.·ha^{-1}·yr^{-1} and 400 equiv.·ha^{-1}·yr^{-1} at the Turkey Lakes watershed just east of Lake Superior (Jeffries et al. 1988; National Atmospheric Deposition Program 1982–1991).

Unlike sulfur, nitrogen emissions in the region began to level off in the early 1980s and were unchanged during the rest of the decade (National Acid Precipitation Assessment Program 1990). The absence of change in emissions likely accounts for the lack of trends in precipitation concentration or deposition (Lynch et al. 1995).

The increase in annual NH_4^+ input (Table 1) concurrent with a decline in winter input (Table 2) during the study could be a response to warmer temperatures (Fig. 10). Air temperatures have been warming in this area since 1950 ($p < 0.001$, $r^2 = 0.28$), and during this study, temperatures increased 0.05°C·yr^{-1} (Fig. 10). Higher temperatures could increase NH_3 emissions from the forest floor during the snowfree season and add to regional NH_4^+ deposition. Expanded agricultural use, however, is still the likely cause.

The percentage decline in total winter precipitation during the study was similar to that in annual precipitation. However, the reduction in winter SO_4^{2-} deposition was about 60% greater than could be accounted for by decreased precipitation amount (Table 2). During the study, more than two thirds of the decrease in precipitation SO_4^{2-} concentration occurred in winter. The change in SO_4^{2-} concentration translates into a reduced deposition of 5 equiv.·ha^{-1} each winter, or most of the SO_4^{2-} deposition decline beyond that accounted for by decreased precipitation.

Higher winter precipitation amounts increase seasonal ion input (Lovett and Kinsmann 1990; Stottlemyer and Troendle 1992). In northern Michigan, the high snowfall zone (>510 cm·yr^{-1}) stretches along the south shore of Lake Superior from Trimountain to Mt. Brockway in Michigan's Keweenaw Peninsula and in the Huron Mountains south of the Keweenaw. The increase in solute loading with <200-m elevation gain at Calumet could also be important. Driscoll et al. (1988) and Lawrence et al. (1988) found that shorter water residence times in upper portions of watersheds reduce the time for melt-water interaction with forest soils.

Within winter, the uniformity of precipitation NO_3^- and SO_4^{2-} inputs up to early March (Figs. 6*e*–6*f*) suggests a common source region. The precipitation NO_3^-/SO_4^{2-} ratio showed little change up to mid-March. In late March, the ratio declined associated with a consistent switch in prevailing wind direction from the WNW to SSE (Stottlemyer and Toczydlowski 1991). The wind shift brings more airborne contaminants from industrial areas in the south (Cadle et al. 1990; English et al. 1986; Semkin and Jeffries 1988), increasing SO_4^{2-} but not NO_3^- input. Late winter precipitation Ca^{2+} and NH_4^+ concentrations and input increased the most relative to weekly input up to peak snowpack (Figs. 6*b* and 6*d*). We attribute the increase to loss of regional snowpack, exposing more land within the region, which could increase dust and NH_4^+ concentrations from agricultural land (Glass and Loucks 1986). The concentration

Fig. 9. Change in (*a*) stream-water discharge and (*b* and *c*) major ion concentration during winter, 1982–1991, Calumet watershed, Michigan. Error bars are slightly offset to avoid overlap. In *b*, the ions are calcium (—), sulfate (---), and HCO_3^- (····). In *c*, the ions are ammonium (—) and nitrate (---).

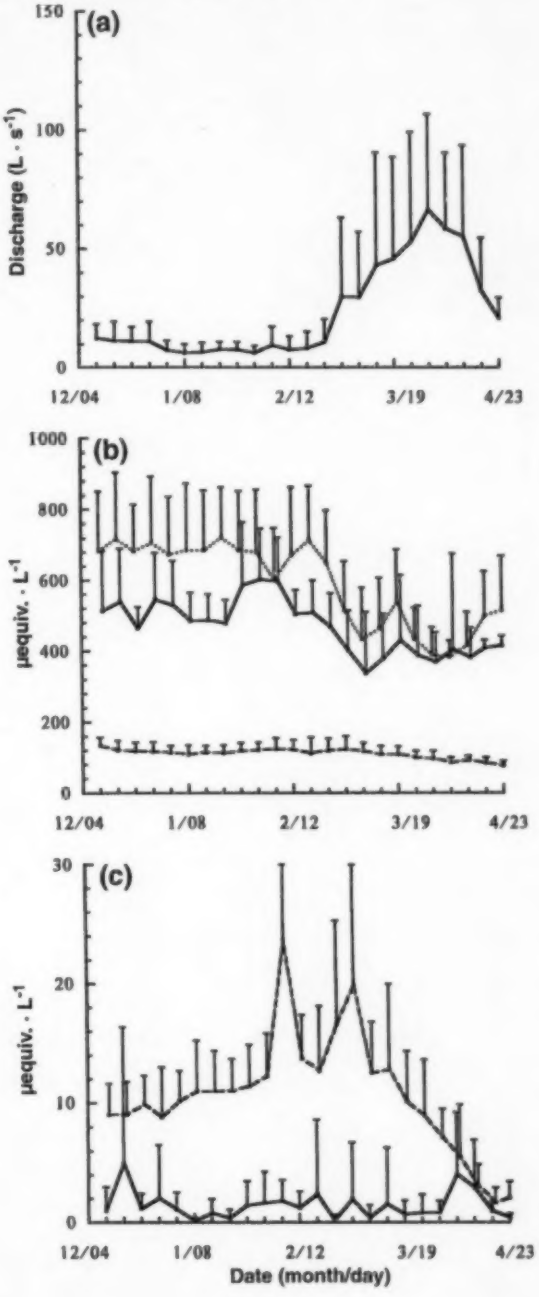

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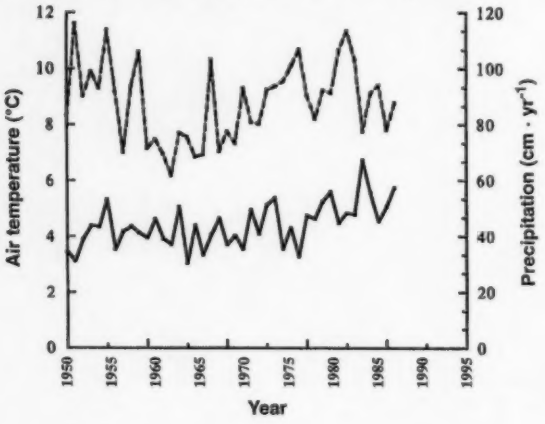
Table 4. Mean weekly stream-water runoff and ion output before peak snowmelt, peak weekly stream-water runoff and ion output, mean maximum snowpack amount and ion content, and cumulative stream-water runoff and ion output up to April 23, 1982–1991, Calumet watershed, Michigan.

	Mean stream-water runoff and ion output	Peak stream-water runoff and ion output	Maximum snowpack amount and ion content	Cumulative stream-water runoff and ion output
Amount, cm	0.4 (0.2–0.7)	3 (1–5)	18 (10–27)	20 (13–33)
Ca^{2+}, equiv.·ha^{-1}	29 (17–53)	162 (66–282)	26 (9–57)	1314 (714–2013)
H^+, equiv.·ha^{-1}	0.003 (0.001–0.006)	0.03 (0.02–0.17)	42 (13–83)	0.2 (0.1–0.4)
NH_4^+, equiv.·ha^{-1}	0.06 (0.003–0.2)	0.5 (1.0–2.4)	19 (2–29)	3.2 (<0.1–5)
NO_3, equiv.·ha^{-1}	0.3 (0.2–0.7)	1.7 (1–7)	36 (16–57)	13 (10–19)
SO_4^2, equiv.·ha^{-1}	5 (3–8)	29 (17–47)	39 (14–99)	220 (136–346)

Note: The range is in parentheses.

Fig. 10. Trends in annual precipitation (---) and mean annual air temperature (—), 1950–1991, National Oceanographic Atmospheric Administration station, Houghton County Airport, 6 km from station C1 in Calumet watershed, Michigan.



increase for all ions was concurrent with the shift in prevailing wind direction and the increase in rain and mixed rain with snow. During the last 4 weeks of snowpack, rain can make up 50% of precipitation at Calumet (Stottlemyer 1987; Stottlemyer and Toczydlowski 1991), and rain has a higher ion-scavenging capacity than snow (Semkin and Jeffries 1988).

Snowpack and snowmelt

Up to snowpack PWE, the patterns of cumulative precipitation and SWE were similar though steadily diverging (Fig. 6*a*). In other studies on this site using snow lysimeters, we found steady snowmelt throughout winter (Stottlemyer and Toczydlowski 1991, 1996). Cumulative snowmelt up to snowpack PWE was similar in both studies. In the present study, at snowpack PWE cumulative snowmelt equalled the difference between cumulative precipitation and SWE, indicating little sublimation to this date.

As with precipitation (Fig. 3), SWE varied with elevation (Fig. 7), but the relative SWE differences with elevation were greater. At all elevations, variation (standard deviation), expressed as a percentage of mean weekly SWE, was uniform up to snowpack PWE. At this time, added variation in watershed SWE occurred because the lowest elevation station (C4) reached snowpack PWE 2 weeks earlier than did station C1. The increase in SWE variation after snowpack PWE primarily reflects yearly SWE differences, the rate of SWE loss after snowpack PWE, and the week of last measurable snowpack (Fig. 8*a*). The pattern of SWE accumulation and loss seemed little influenced by total winter precipitation. For example, cumulative winter precipitation for 1983 and 1986 differed by <10%, but the trends in SWE were divergent (Fig. 8*a*) (Stottlemyer and Rutkowski 1990).

Unfrozen soils and midwinter thaws appear to be the rule in this region (Jeffries 1990), though there are exceptions south of the Lake Superior snow belt (Cadle et al. 1984, 1986). At Calumet, unfrozen soils provide convective heat flux to the base of the snowpack, which metamorphoses snow crystals and results in steady loss of SWE well before peak snowmelt (English et al. 1986; Stottlemyer and Toczydlowski 1991). Freeze–thaw cycles are also common (Stottlemyer and Toczydlowski 1996) and contribute to variation in snow-melt rates throughout winter (Colbeck 1981), as can the movement of meltwater along ice lenses within the snowpack (English et al. 1986). At Calumet, rain and mixed rain–snow inputs, especially during the last month of snowpack, increase snowmelt rates and often reduce snowpack chemical content (Jones 1987; Stottlemyer 1987). The physical and chemical interaction of rain with the snowpack and the rapid and variable redistribution of solutes within the snowpack would also increase variability in snowmelt chemistry (Fig. 6).

Up to snowpack PWE, the pattern of snowpack H^+, NO_3^-, and SO_4^{2-} content more closely resembled cumulative precipitation input than change in SWE (Figs. 6*a*, 6*c*, 6*e*, 6*f*). Adsorption of dry deposition might also explain some of the trend in snowpack ion content. For example, with warming snowpack temperatures, especially at the surface, there is an increase in adsorption of SO_2, which, with sufficient oxidant, is transformed into SO_4^{2-} (Conklin et al. 1993). Similarly, the deposition velocity of HNO_3^- to snowpack surfaces is high relative to other species and could supplement wet precipitation NO_3^- input (Cadle et al. 1986). The increase in snowpack NH_4^+ content (Fig. 6*d*) followed the trend in SWE. In contrast with the elevated winter atmospheric SO_2 concentrations in northern Michigan and high HNO_3^- deposition velocities, NH_4^+ (and NH_3) particulate concentrations and deposition velocities are low (Cadle et al. 1986). These factors, coupled with large midwinter snowmelt NH_4^+ loss, could account for the lower snowpack NH_4^+ content relative to precipitation input. The trend in snowpack Ca^{2+} content also followed SWE up to early February (Fig. 6*b*). After that, snowpack Ca^{2+} content increased more rapidly than SWE or cumulative precipitation Ca^{2+} input.

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The Ca^{2+} increase suggests snowpack adsorption of dust inputs concurrent with loss of the regional snowpack (Glass and Loucks 1986) or increased leaching of debris within the snowpack (Dewalle 1987; Hornbeck and Likens 1974). Variation in weekly snowpack Ca^{2+} and NH_4^+ content, as a percentage of the mean, increased several weeks before snowpack PWE (Figs. 6*b* and 6*d*, 8*b* and 8*d*) but precipitation inputs remained steady. Cadle et al. (1986) estimated that dry deposition of Ca^{2+} and Cl^- in northern Michigan is about one third of total atmospheric input, larger than for H^+, NH_4^+, or SO_4^{2-}, but overall much smaller than dry deposition nearer urban centers.

Up to snowpack PWE, the snowpack ion content averaged 66 ± 14% of cumulative precipitation inputs, and estimated snowmelt ion loss was 45 ± 9% of cumulative precipitation inputs. The difference indicates dry deposition inputs to the snowpack equal to >10% of cumulative precipitation inputs up to this date. Bulk precipitation collectors do capture more dry deposition than "wet" or "event" precipitation collectors, but because of their shape and surface texture, they are not a reliable measure of dry deposition input (Cadle et al. 1986; Jeffries 1990).

The presence or absence of frozen soils is a major factor regulating the flow path, and therefore chemical composition, of snowmelt after leaving the snowpack. When soils are frozen, the amount and timing of snowmelt is altered (Cadle et al. 1984, 1986; Semkin and Jeffries 1988), and watershed ion export can be changed because snowmelt is isolated from soils (Pierson and Taylor 1985). Frozen soils may also increase watershed export of limiting nutrients such as NO_3^- from enhanced soil nitrification (Likens et al. 1977) or reduced biological immobilization (Lewis and Grant 1980). Conversely, unfrozen soils permit percolating snowmelt to interact with soils chemically. Unfrozen soils improve the opportunity for limited soil biological activity, such as microbial immobilization of nitrogen, which accounts for the strong ecosystem retention of snowmelt NH_4^+ and NO_3^- at Calumet. Unfrozen soils permit greater desorption and mineralization rates, which can increase ecosystem export of ions such as SO_4^{2-} (Foster et al. 1989; Hazlett et al. 1992; Stottlemyer and Toczydlowski 1996).

Differential snowpack ion loss has been observed for decades (Bales et al. 1989; Bowman 1992; Johannessen and Henriksen 1978; Stottlemyer and Toczydlowski 1991). Our results indicate that NH_4^+ is the ion first lost in the elution sequence, which differs somewhat from results in previous studies where SO_4^{2-} is generally the ion eluted first. However, reproducible elution sequences have been difficult to obtain in real snowpacks because of varying experimental conditions and different ion solubilities in ice (Davies et al. 1987).

Soil water and stream water

Annual runoff at Calumet, as a percentage of precipitation, was average for the region. Runoff from 20 basins in the Turkey Lakes watershed ranges from 28 to 63% of precipitation (Nicholson 1988). At Calumet, the percentage of annual runoff that occurred in winter and in the month of April matched values from both the long-term record at Hubbard Brook, N.H. (Likens and Bormann 1995), and the Turkey Lakes watershed.

Unlike some other studies in northern watersheds (Likens and Bormann 1995), we did not find a correlation between annual precipitation and stream-water runoff ($p = 0.13$). Similarly, evapotranspiration, as estimated by the difference between precipitation and runoff, was not independent of precipitation but highly related ($p < 0.001$, $r^2 = 0.84$). The gradual decrease in annual stream-water runoff could reflect a decline in groundwater contributions, but we have no direct measure of this. In summer, the hydrograph rarely responds to precipitation inputs, suggesting that little precipitation reaches the stream. In winter, we operate two shallow (2 m deep) wells near stations C3 and C1 that measure change in soil water level above the nearly impervious ortstein layer. Winter stream-water runoff at the weir is highly related ($p < 0.001$, $r^2 =$ 0.5–0.9) to change in soil water level in these wells, suggesting that most winter runoff is from water above this cemented soil layer (Stottlemyer and Toczydlowski 1991).

As in other snow-dominated regions, spring melt is a significant hydrologic event at Calumet. During early winter and midwinter, soil water ion concentrations were high because of the long contact period with the soil matrix (Stottlemyer and Toczydlowski 1996). With initial snowmelt, this "older" water can be forced out (Abrahams et al. 1989; Campbell et al. 1995; Rascher et al. 1987), resulting in a small pulse or increase in stream-water ion concentration, as seen in early March most years (Figs. 9*a* and 9*c*). Dilution and movement of ions from soil exchange sites result in rapid change in stream-water ion concentration. While overland flow amounts are small (<10%) at Calumet (Stottlemyer and Toczydlowski 1991), during peak snowmelt, the soil water level is near or at the soil surface for up to a week. The more rapid movement of snowmelt through near-surface organic soils and its reduced residence time decrease stream-water C_B concentration. Dilution is the dominant factor reducing stream-water ANC. In other studies (Barry and Price 1987), dilution accounts for similar reductions (>90%) in ANC. While stream-water ANC is reduced during peak runoff, turbidity and concentrations of dissolved organic carbon and total organic N and P increase (Stottlemyer and Toczydlowski 1991).

At Calumet, soil exchange retains nearly all H^+ in snowmelt, and stream-water H^+ output at peak runoff is <1 equiv.·ha^{-1} (Fig. 6*c*). More than 99% of precipitation H^+ input is retained by the ecosystem. Similarly, adsorption, biological uptake, and transformation reduce NH_4^+ output and concentrations to minimal levels throughout winter except immediately after snowpack establishment (Figs. 6*d*, 9*c*). Greater than 95% of precipitation NH_4^+ input is retained.

Annual and winter stream-water NO_3^- and SO_4^{2-} output showed no significant trend despite declines in inputs that approached 50% for SO_4^{2-} during the decade (Table 1). Precipitation and snowmelt anions appear more likely than cations to have their relative proportions changed by soil reactions before reaching the stream (Tranter et al. 1987). For NO_3^-, we attribute the lack of an output trend to soil processes modifying snowmelt input. For example, studies using the difference between precipitation and soil-water NO_3^- isotope ratios have found retention of atmospheric NO_3^- inputs by soil microbial immobilization concurrent with ecosystem NO_3^- loss in soil water (Durka et al. 1994). Stream-water NO_3^- output could reflect the net effect of trends in winter soil processes rather than snowmelt inputs. At Calumet, with the beginning of the major snowmelt period in March, stream-water NO_3^- concentrations begin to decline, reaching the level of detection shortly after the end of snowpack (Fig. 9*c*). The decline indicates

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strong ecosystem retention during and immediately after peak stream-water runoff. More than 80% of winter precipitation NO_3^- input was retained in the watershed even though about half of the snowmelt NO_3^- was released before snowpack PWE when biological immobilization is minimal.

For SO_4^{2-}, the absence of much decline in stream-water concentration during peak runoff likely reflects soil desorption (Harrison et al. 1989; Jeffries 1990; MacDonald et al. 1994). Studies with a variety of forest soils show that up to two thirds of adsorbed SO_4^{2-} can be desorbed by percolating water (Harrison et al. 1989). In areas such as Calumet that historically experienced high levels of SO_4^{2-} deposition, large reservoirs of SO_4^{2-} in forest soils may be present. Soil SO_4^{2-} desorption seems the only process with the capacity to buffer change in stream-water SO_4^{2-} concentration with such large variation in stream-water discharge (Figs. 9*a*, 9*b*).

In a separate study of soil-water chemistry at Calumet (Stottlemyer and Toczydlowski 1996), we found an increase in forest-floor percolate SO_4^{2-} concentration relative to snowmelt concentrations. Desorption, some mineralization, and likely release of aerosol deposition from the previous snowfree season could account for the SO_4^{2-} increase (Lindberg et al. 1986; Reuss and Johnson 1986). In that study, forest-floor percolate dissolved organic carbon concentrations also increased sharply, and there was a significant correlation between SO_4^{2-} and dissolved organic carbon concentrations. Elevated dissolved organic carbon concentrations could partly account for the increased percolate SO_4^{2-} concentration because dissolved organic carbon reduces soil SO_4^{2-} adsorption (Vance and David 1992).

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Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts

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Abstract: Groups of Atlantic salmon (*Salmo salar*) smolts were exposed to full-strength seawater for 1, 6, 12, 24, 48, 96, and 144 h or were left in fresh water (control). Their physiological performance was monitored as changes in levels of plasma chloride, hematocrit, and muscle water content. At 12 and 24 h, smolts showed a transient increase in plasma chloride levels concurrent with a reduction in hematocrit, indicating a significant osmotic stress during this period. After 48 h, values fell to normal resting levels in seawater and no further changes were observed. In a second experiment, similar groups of smolts were exposed to seawater for 0 (control), 12 , 24, 48, and 96 h and then confronted with predatory cod (*Gadus morhua*). Groups exposed to seawater for 12 and 24 h showed a change in antipredator behaviour, i.e., reduced escape distance and schooling behaviour, and suffered significantly higher predatory mortality than control fish or groups fully acclimated to seawater. These results suggest that Atlantic salmon smolts that suffer physiological stress while transferring from fresh water to the sea may also be exposed to increased predation as a consequence of less effective antipredator behaviour.

Résumé : Des groupes de smolts de saumon atlantique (*Salmo salar*) ont été exposés à de l'eau de mer à pleine concentration pendant 1, 6, 12, 24, 48, 96 et 144 h ou ont été laissés en eau douce (témoins). Leur performance physiologique a été mesurée par les changements de la concentration de chlorure dans le plasma, de l'hématocrite et de la teneur en eau des muscles. Au bout de 12 et 24 h, les smolts présentaient une augmentation temporaire de la concentration de chlorure dans le plasma en même temps qu'une réduction de l'hématocrite, ce qui indique un important stress osmotique pendant cette période. Au bout de 48 h, les taux sont tombés aux niveaux normaux de repos dans l'eau de mer, et aucune autre modification n'a été observée. Dans une deuxième expérience, des groupes similaires de smolts ont été exposés à de l'eau de mer pendant 0 (témoins), 12, 24, 48 et 96 h, puis confrontés à des morues (*Gadus morhua*) prédatrices. Les groupes exposés à l'eau de mer pendant 12 et 24 h ont présenté un changement dans le comportement anti-prédateur, c'est-à-dire une réduction de la distance de fuite et du comportement grégaire, et ont subi une mortalité par prédation nettement plus élevée que les poissons témoins ou les groupes pleinement acclimatés à l'eau de mer. Ces résultats permettent de penser que les smolts de saumon atlantique, qui subissent un stress physiologique lors du passage de l'eau douce à l'eau de mer, peuvent aussi être exposés à une prédation plus forte par suite de la moindre efficacité de leur comportement anti-prédateur.
[Traduit par la Rédaction]

Introduction

An anadromous life-style is common among salmonid fish. This strategy involves large-scale movements from fresh water to the sea within a short period of time (for reviews see Baker 1978; McDowall 1988). During the smolt migration period, predators assemble in estuaries and adjacent areas to reap their harvest of these migrants. Atlantic salmon, *Salmo salar* L., spend a year or more in fresh water before migrating to the sea as smolts (see Hoar 1988; Wedemeyer et al. 1980). During seaward migrations, smolts in the Baltic Sea are vulnerable to predation by burbot, *Lota lota*, pike, *Esox lucius*, and gulls and terns (Larsson 1985; Valle 1985). On the Norwegian coast, migrating salmon smolts are preyed upon by cod (*Gadus morhua*), saithe (*Pollachius virens*), pollack (*Pollachius pollachius*), and gulls (Gunnerød et al. 1988; Hvidsten and Lund 1988; Hvidsten and Møkkelgjerd 1987; Reitan et al. 1987; Skilbrei et al. 1994) and field studies indicate that mortality from predation during seaward migration may reach 20–25% (Hvidsten and Møkkelgjerd 1987; Hvidsten and Lund 1988).

When salmonid smolts are migrating from fresh water to seawater, the change in external salinity subjects them to physiological (osmotic) stress (e.g., Eddy 1981; Hoar 1988). In laboratory experiments under controlled conditions typical consequences of hyperosmotic stress include increased plasma chloride concentrations (Järvi 1990; McCormick et al. 1989; Sigholt and Finstad 1990), muscle dehydration (Blackburn and Clarke 1987; Sigholt and Finstad 1990), and a reduced hematocrit level (Bakke et al. 1991; Bath and Eddy 1979). Smolts are normally not completely adapted to seawater until after 4–6 days (Prunet and Boeuf 1985). The presence of predators may itself induce a physiological stress response in fish, and Atlantic salmon smolts exposed to predatory cod in an experimental situation show increased levels of plasma chloride (Järvi 1990). When smolts are migrating from fresh water to seawater, osmotic stress, together with the presence of predators, is thought to induce a cumulative stress response (Järvi 1989*a*, 1990), which may inhibit the normal, adaptive, antipredator behaviour of the fish and increase the risk of predation (Järvi 1989*b*).

Atlantic salmon react to predators by fleeing, freezing, and schooling (Jakobsson and Järvi 1977; Järvi 1989*b*). Any given

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Table 1. Experimental protocol for creating groups with different acclimation time (hours) in experiment 2.

Hours in seawater	Time spent in first tank (h)		Time spent in second tank (h)		Total acclimation time
	Freshwater	Seawater	Freshwater	Seawater	
0	72	0	24	0	96
12	72	0	12	12	96
24	72	0	0	24	96
48	48	24	0	24	96
96	0	72	0	24	96

reaction may bring the benefit of escape but may also have costs. Optimal antipredator behaviour may be determined by a balance between the costs of reacting to the predator and those of remaining passive (Ydenberg and Dill 1986). Osmotic stress may influence this balance through the increased cost of maintaining homeostasis during seawater acclimation, leaving less energy available for swimming. For instance, rapid flight demands high oxygen consumption, leading to accumulation of lactate in the blood (Forsman and Virtanen 1989; Tufts et al. 1991), which decreases subsequent swimming capacity (Brett 1964). This could lead to stressed fish suffering a higher risk of predation than unstressed fish. We might thus expect osmotically stressed fish to allow a predator to approach closer before trying to escape, as an elevated lactate level could increase the risk of being captured in later attacks. Likewise, because of the costs of coordinated swimming we should expect unstressed smolts to demonstrate more schooling than smolts experiencing osmotic stress.

This study attempted to determine whether there is a correlation between osmotic stress, antipredator behaviour, and predatory mortality in Atlantic salmon smolts. Osmotic stress influences several physiological parameters, and the interaction of different factors may determine the physiological state of the fish. We recorded a number of parameters to identify those that could best explain the expected changes in behaviour and mortality in osmotically stressed smolts. We performed two series of experiments. In the first, physiological parameters in smolts exposed to seawater for different periods were recorded. In the second, smolts with the same exposures to seawater were confronted with predatory cod, and their antipredator behaviour and mortality were studied. As the physiology of smolts lost to predators was not known, data on such parameters as size, growth rate, and sex were used to provide information about their physiological and morphological status, permitting a comparison to be made between successful and unsuccessful smolts.

Materials and methods

General

The experiments were carried out in May 1990 outdoors at the research station of the Norwegian Institute for Nature Research (NINA) at Ims (southwestern Norway). The fish were offspring of wild brood stock caught in the River Imsa and had been reared outdoors under ambient light and temperature conditions in a 72-m^2 tank supplied with fresh water from the river until smolting at age 2+. During this period the fish were fed ordinary commercial dry diet, according to temperature and fish size. The fish were size graded and individually tagged on 8–10 April (Carlin tags, McAllister et al. 1992). Length and weight were as follows (mean ± SD): 8–10 April, 15.2 ± 1.0 cm, 31.7 ± 6.5 g; 12–16 May, 17.3 ± 1.3 cm, 37.9 ± 8.3 g (N = 1200). A 96-h, 33‰ seawater challenge test was done before the experiments (2 May) to describe the physiological status of the smolts (N = 20). During this test period normal behaviour was observed and the fish showed clear morphological characteristics of smoltification, i.e., loss of parr marks, body silvering, dark edge of the dorsal and caudal fins, loose scales, and low condition (K) factor (0.88 ± 0.1, Gorbmann et al. 1982).

Two independent experiments were conducted during May 1990. Experiment 1 was performed between 6 and 15 May, coinciding with the annual natural smolt migration period in the river Imsa during the month of May, to assess the level of osmotic disturbance in smolts exposed to seawater for various lengths of time. Experiment 1 started when the first descending smolts were captured in a Wolf trap. The degree of osmotic stress was measured as changes in plasma Cl^- levels, hematocrit value, and rate of muscle dehydration. In Experiment 2, carried out between 12 and 31 May, a relationship was established between time of seawater acclimation, antipredator behaviour, and risk of predation by cod. Neither smolts nor cod were used more than once in any experiment.

Experiment 1

To study physiological stress on salmon smolts caused by exposure to seawater, eight groups of 15 smolts were placed in each of eight black circular plastic tanks (110 l, height 40 cm), initially supplied with running (2 L/min) natural fresh water (mean temperature in May was 13.9 ± 1.82°C, O_2 > 85%) from the River Imsa. All groups were kept without food in the tanks for 144 h. During this time the different groups were exposed to either fresh water (control) or running seawater (mean temperature in May was 8.0 ± 0.2°C) for 1, 6, 12, 24, 48, 96, or 144 h prior to sampling. The change from fresh water to seawater was obtained by changing the water supply to the tanks. The salinity rose to that of full-strength seawater (33‰) within 50 min. The change in temperature paralleled the changes that wild smolts experience.

Blood and tissue samples were taken from all groups after 144 h. The smolts were removed from the tank and killed by a blow to the head. Blood was sampled from the caudal vein. Samples of white muscle tissue were removed from the peduncle with a cross section in the tail (approximately 1 cm^3). The water content of tissue samples was determined as the difference between wet and dry weight (determined after 24 h at 70°C). A subsample of blood was used for hematocrit determination, while the remainder was centrifuged to separate plasma and blood cells. Plasma levels of Cl^- (mM) were determined in duplicate samples of 20 µL of plasma in a Radiometer CMT 10 titrator.

Experiment 2

Two experimental arenas were constructed outdoors, using ordinary square rearing tanks (area 12 m^2, height 1 m, water level 40 cm) continuously supplied with seawater and with no cover for the smolts. The water exchange rate was approximately 20 L/min. Between the two tanks there was a tower for observations of behaviour. Six replicate trials were made for each acclimation group in the period 12–31 May. To increase the number of trials within a given time period each trial consisted of two steps. First, 15 smolts were kept without food for 72 h in a tank similar to that used in experiment 1. The smolts were then carefully transferred to identical tanks inside the arenas for a further 24 h of acclimation. The bottom of each new tank was replaced by a metal plate and a rope, connecting the tank to the observation tower, making it possible to lift the tank and release the smolts in the experimental arena. The total time spent in the tanks was always 96 h, with seawater exposure times ranging from 0 to 96 h (0, 12, 24, 48, and 96 h corresponding to time of seawater exposure in experiment 1, Table 1).

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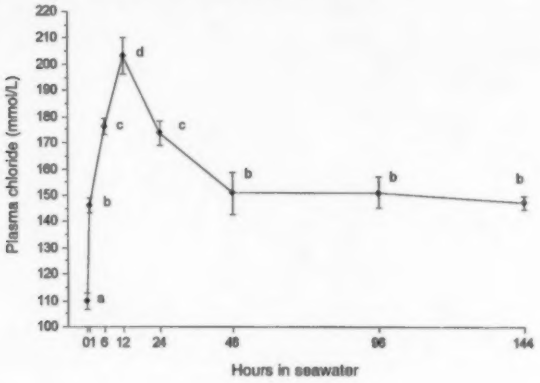
Fig. 1. Mean plasma chloride levels (± SE, $N = 15$) of smolts relative to exposure time to seawater in experiment 1. The first treatment (0 h) corresponds to the freshwater group (control). Significant differences between groups are indicated by different letters ($p < 0.05$, Student–Newman–Keuls test).



Before the smolts were transferred to the acclimation tank inside the arena, four wild-caught cod were placed in the arena. The cod were not visible to the smolts inside the acclimation tank. The cod were bought from local fishermen 1 month before the start of the experiment (mean weight = 1.47 ± 0.54 kg, $N = 120$). As with the smolts, each cod was used only once. At the end of the seawater exposure period, the acclimation tank was gently removed from the arena, leaving the 15 smolts exposed to the cod.

With this experimental setup it was possible to conduct two tests each day. To eliminate the effect of changes in physiological status over time a new acclimation group was tested each day in a 5-day cycle in each arena (12 May, two trials with control group; 13 May, two trials with 12-h group, and so on). There were no significant differences in smolt behaviour (schooling behaviour and escape distance) between the trials in each acclimation group (analysis of variance (ANOVA)) and no change in smolt morphology (body silvering and fin status).

All observations were made in the morning between 06:00 and 08:00. Smolt behaviour was continuously observed and recorded using a camera and videocassette recorder (VCR) from the observation tower for 1 h. After the completion of a trial, all smolts and cod were caught. The stomach of each cod was removed and opened, and the smolts it had eaten were taken out. The length (postorbital-hypural and fork length), weight, growth rate, K factor, and pectoral fin status (length between base of fin and fin tip, indicating ability to manoeuvre) of both eaten and living smolts were recorded. The ductus spermaticus (male) and gonads (female) were used to determine sex. The relative growth rate (RGR) was calculated according to the following formula:

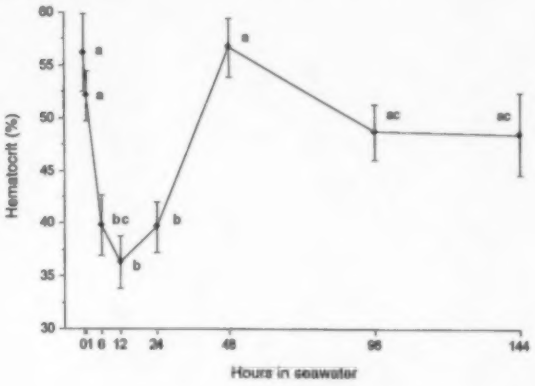
$$\mathrm{RGR} = (W_2 - W_1)/(W_1(T_2 - T_1))$$

(Busacker et al. 1990), where W_2 and W_1 are mean weights at day T_2 (termination date) and T_1 (tagging date), respectively. The K factor was calculated using the formula $K = 100\ W/L^3$, where W is the weight and L is the corresponding fork length.

The schooling behaviour and escape distance of smolts were analysed from videotapes. Pilot tests showed that the smolts behaved representatively within half a minute of release into the experimental arena. To eliminate the immediate effect of confusion the first minute after release in each trial was excluded when analysing the videotapes.

Numbers of single and schooling smolts were recorded on seven occasions during each trial (after 5, 10, 20, 30, 40, 50, and 60 min). The fish were classified as schooling when at least two fish swam in the same direction at a distance of less than two body lengths (Pitcher and Parrish 1993). The distance at which a smolt reacted to the predator was defined as the escape distance (Jakobsson and Järvi 1977). Escape distance of single and schooling smolts was estimated when a predator approached from the front (45° + 45°, i.e., cranial-caudal axis), from the side, or from behind (45° + 45°, i.e., cranial-caudal axis). Because each individual smolt might react similarly on several occasions, causing intra-individual ties, only the mean escape distance and mean number of schooling smolts in each trial were used in the statistical analyses.

Fig. 2 Mean hematocrit levels (± SE, $N = 15$) of smolts in relation to exposure time to seawater in experiment 1. (See Fig. 1 for further details.)



Statistical procedures

All statistical analyses and statistical plots were developed using the software Excel 3.0a (Microsoft) and Statistica 3.0a (StatSoft). In experiment 1, all analyses were based on ANOVA and a Student–Newman–Keuls post-hoc test. In experiment 2 a Kruskal–Wallis ANOVA (nonparametric) and a Mann–Whitney U test were used when the data diverged from the normal distribution. A linear regression was used to identify the correlations between physiological and behavioural parameters, while a multiple regression was used to determine whether sex, growth rate, K factor, length, and pectoral fin status were significantly correlated with mortality rate.

Results

Experiment 1

Changes in physiological status during seawater acclimation

The observed mean plasma Cl^- levels were influenced by exposure to seawater ($p < 0.001$, ANOVA, Fig. 1). Plasma Cl^- levels of smolts exposed to seawater increased rapidly from 110.0 mM (freshwater controls) to a peak after 12 h of exposure (203.2 mM, $p < 0.05$, Student–Newman–Keuls post-hoc test) with a subsequent significant decrease at 24 (173.5 mM, $p < 0.05$) and 48 h (150.8 mM, $p < 0.05$). Plasma Cl^- levels showed no further change, remaining at approximately 150 mM at 96 and 144 h. All Cl^- levels observed after seawater exposure were higher than those of freshwater controls ($p < 0.05$).

Significant reductions in hematocrit value and muscle water content were observed after seawater exposure (Figs. 2 and 3, $p < 0.001$, ANOVA). Mean hematocrit value decreased from 56.2% (freshwater controls) to a low of 36.3% following

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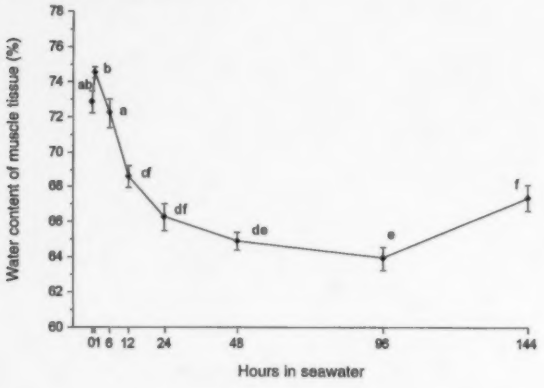
Fig. 3. Mean water content of muscle tissue (± SE, $N = 15$) of smolts in relation to exposure time to seawater in experiment 1. (See Fig. 1 for further details.)



12 h of seawater exposure ($p < 0.05$, Student–Newman-Keuls post-hoc test), while muscle water content fell from 72.8 to 68.6% during the same period ($p < 0.05$). Between 12 and 48 h the mean hematocrit value increased significantly, reaching levels comparable to those observed in freshwater controls (approximately 50%). Muscle dehydration continued for the first 96 h in seawater, reaching a low of 63.9% ($p < 0.05$). A subsequent increase in water content was observed between 96 and 144 h exposure to seawater (67.3%, $p < 0.05$), but this level was still significantly lower than that of freshwater controls. The plasma chloride level during seawater acclimation was correlated with the hematocrit value ($r^2 = 0.77$, $p < 0.01$). There was no correlation between plasma chloride level and muscle dehydration ($r^2 = 0.05$, $p = 0.59$).

Experiment 2

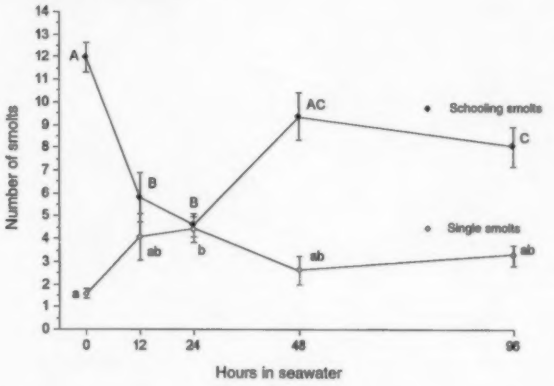
Schooling behaviour

Schooling behaviour was influenced by seawater exposure ($p < 0.001$, ANOVA, Fig. 4). The median number of schooling smolts was significantly higher in the control group than in the groups kept for 12 and 24 h in seawater ($p < 0.05$, Student–Newman–Keuls post-hoc test). For seawater-exposed smolts a consequence was a significant increase in the median number of single smolts in the same period ($p < 0.05$, Student–Newman–Keuls post-hoc test). Between 24 and 48 h, the median number of schooling smolts increased significantly ($p < 0.05$), with a corresponding decrease in the number of single smolts. Between 48 and 96 h of acclimation the schooling behaviour stabilized; however, it remained significantly ($p < 0.05$) below that of the control group. The median number of schooling smolts during seawater acclimation correlated with both the plasma chloride level ($r^2 = 0.77$, $p < 0.05$) and the hematocrit value ($r^2 = 0.81$, $p < 0.05$) recorded in experiment 1. There was no correlation between schooling behaviour and muscle dehydration ($r^2 = 0.07$, $p = 0.63$).

Escape distance

After 12 and 24 h of exposure to seawater, single smolts showed a significant reduction in mean escape distance when approached by the predator from in front or behind ($p < 0.05$, Mann–Whitney U test, Fig. 5A). The escape distance for single smolts attacked from either angle increased significantly between 24 and 48 h of seawater exposure ($p < 0.05$), reaching mean values similar to those of freshwater controls. No further changes were observed between 48 and 96 h. The overall escape distance for smolts attacked from the side did not change significantly at any length of exposure to seawater and, except for 24-h attacks from behind, was significantly higher than for attacks from other angles at 12 and 24 h of exposure to seawater ($p < 0.05$).

Fig. 4. Schooling activity (median number of schooling and single smolts) in relation to exposure to seawater in experiment 2. The first treatment (0 h) corresponds to the freshwater group (control). For each treatment, six trials were performed, each involving 15 smolts and four cod. Significant differences between groups are indicated by different letters (schooling smolts: $p < 0.05$, Student–Newman–Keuls test; single smolts: $p < 0.05$, Mann–Whitney U test).



The escape distance of schooling smolts was generally greater than that of single smolts (compare Figs. 5A and 5B). After 12 h of exposure to seawater a significant reduction in escape distance was observed for schooling smolts attacked from behind ($p < 0.05$, Mann–Whitney U test, Fig. 5B), with a subsequent increase after 24 h. No further changes were observed in escape distances for smolts attacked from behind or the front. Like the results for single smolts, escape distance for attacks from the side was higher than for other angles. Except for an increase after 48 h ($p < 0.05$), no significant changes in escape distance for attacks from the side were observed after any period of exposure to seawater.

Predation mortality

The number of smolts captured by cod was related to the time they had spent in seawater ($p < 0.01$, ANOVA, Fig. 6). Smolts exposed to seawater for 12 and 24 h were caught significantly more often than smolts that had spent either a shorter or a longer time in seawater. Predation mortality was higher in single than schooling smolts ($p < 0.05$, Student's t test, Fig. 7) and 80% of the smolts that were caught were single.

Mortality from predation was correlated with the median number of schooling smolts ($r^2 = 0.92$, $p < 0.01$) as well as with the plasma chloride levels ($r^2 = 0.79$, $p < 0.03$) and hematocrit value ($r^2 = 0.86$, $p < 0.02$) recorded in experiment 1. There was no correlation between predation mortality and

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Fig. 5. Escape distance for single (A) and schooling (B) smolts in relation to exposure to seawater in experiment 2 ($p < 0.05$, Mann–Whitney U test, see Fig. 4 for further details).

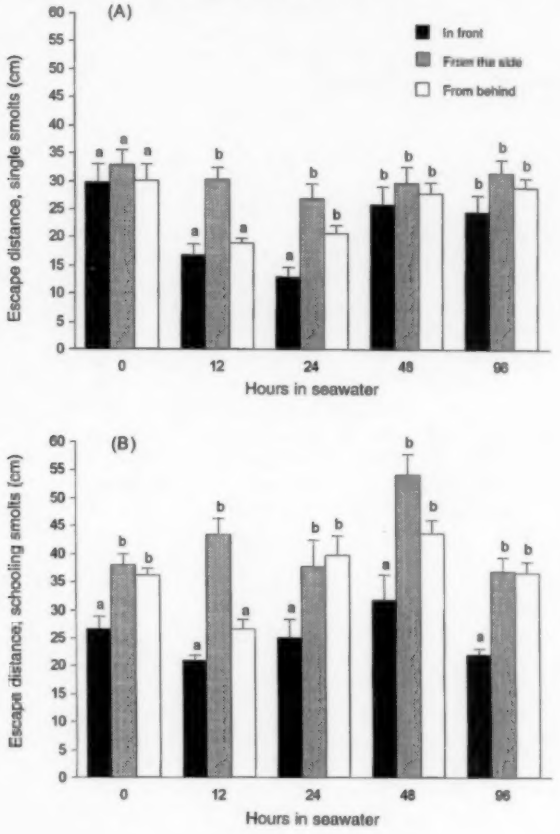

Fig. 6. Total number of smolts eaten relative to time of exposure to seawater in experiment 2 ($p < 0.05$, Student–Newman–Keuls test, see Fig. 4 for further details).

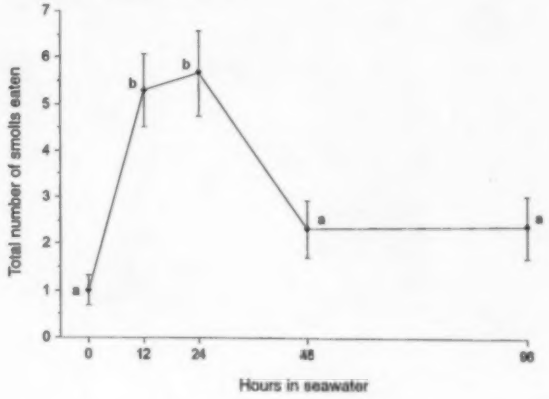

Table 2. Total number of eaten and surviving smolts by sex and predation rate (%) of males and females in experiment 2.

Sex	Total number of smolts (N)	Surviving smolts (N)	Eaten smolts (N)	Predation rate (%)
Male	200	144	56	28.0
Female	211	170	41	19.4
Sum	411	314	97	—

muscle dehydration of smolts in the two experiments ($r^2 = 0.05$, $p = 0.5$).

Morphological characteristics and mortality rate

Significantly higher mortality rates were observed in male than female smolts: 28% as opposed to 19.4% ($p < 0.05$, multiple regression, Table 2). Mortality decreased with increasing growth rate ($p < 0.05$). The relative growth rates of surviving males and females were estimated at 0.41 and 0.33%/day, while the corresponding estimates for males and females eaten were 0.27 and 0.26%/day, respectively. No relationships were observed between mortality and length or weight (surviving smolts: length = 17.4 ± 1.2 cm, weight = 38.4 ± 8 g; eaten smolts: length = 17.2 ± 1.4 cm, weight = 36.3 ± 9 g), nor between mortality and K factor and pectoral fin status.

Discussion

This study demonstrates a correlation between physiological changes and changes in the antipredator behaviour and mortality rate of smolts exposed to predators. Physiologically stressed smolts showed a weaker reaction towards predators and were more often preyed upon, suggesting that physiological stress significantly influenced behaviour and may have been important in determining the outcome of interactions between salmon smolts and their predators.

As the physiology of consumed smolts could not be studied, two separate experiments were performed; the first recorded the effects of seawater exposure on physiological parameters, and the second the effects of osmotic stress on antipredator behaviour and mortality rate. One problem associated with this experimental design was that smolts in the second experiment, in addition to osmotic stress, experienced two stressors, i.e., the presence of predators and some additional handling that might have increased their stress response (Järvi 1990). This means that the osmoregulatory problems of smolts exposed to predators could be more serious than revealed by experiment 1. However, in experiment 1 the fish also showed clear signs of being physiologically stressed after 12 and 24 h of exposure to seawater and the cumulative stress level in experiment 2 could not have been markedly higher as no mortality was observed except from predation. In addition, the development with time of seawater exposure should not be influenced by any cumulative stress response, and the correlations between physiology, behaviour, and mortality should thus be valid. Another problem was that the time delay between the two experiments may have caused differences in smolt quality. In the River Imsa the annual smolt migration lasts for 3 weeks (Hansen and Jonsson 1985; Jonsson and Ruud-Hansen 1985), and our experiments (6–31 May) were timed to coincide with this migration, with maximum seawater migration during the overlap period between experiments 1 and 2. In experiment 2 the effects of possible changes in smolt quality over time were minimized by permutating the order of

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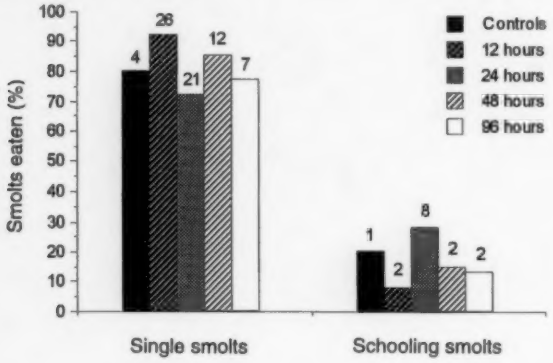
Fig. 7. Percentage distribution of total predation on single and schooling smolts in experiment 2. *N* is the number of observations in each acclimation group.



testing. No changes in smolt behaviour or morphology were observed over time. Smolt quality in the experiments was thus regarded as equal.

Several physiological parameters were recorded in this study to arrive at an indication of the factors that caused the changes in antipredator behaviour. Plasma chloride concentration and hematocrit level showed the highest correlations with behaviour and mortality, while the changes in muscle water content showed a time lag, with a high degree of dehydration still evident when the smolts had returned to their normal behaviour. The correlations alone do not tell us whether plasma chloride or hematocrit level is most important for the behavioural changes. Further, these factors are related. An increase in the plasma chloride concentration has several physiological implications, one of which is an increase in blood osmolarity (Finstad et al. 1988). Increased concentrations of plasma ions may lead to a shrinkage of the erythrocytes (Bath and Eddy 1979; Blackburn and Clarke 1987) with a resulting reduction in erythrocyte volume. This may explain the observed decrease in hematocrit after 12 and 24 h of exposure to seawater. A decrease in hematocrit is related to a decrease in the oxygen-carrying capacity of the erythrocytes (Nikinmaa and Soivio 1979; Virtanen and Oikari 1984). A reduction in oxygen-carrying capacity is known to affect the oxygen concentration of muscle tissue, reducing swimming capacity and shifting the fish to anaerobic metabolism and lactate production (Heisler 1980). An escape sequence of osmotically stressed fish would thus lead to accumulation of lactate in muscle and prolonged recovery time (Brett 1964), thereby increasing the risk of being captured by predators. A decrease in oxygen capacity and swimming performance during seawater acclimation (Brauner et al. 1992, 1994) might therefore represent a direct physiological constraint on the behavioural options of smolts towards predatory attacks.

The behaviour of osmotically stressed smolts changed, with decreased escape distance and a lower tendency to school. After 12 and 24 h of seawater exposure, smolts showed a reduction in mean escape distance when approached from behind (both single fish and schools) and from the front (single fish), whereas the escape distance for attacks from the side was greater than for attacks from other angles and did not change with time of exposure to seawater. Escape reactions towards predators approaching from the front may have high costs, as escape requires turning before fleeing, making the fish more vulnerable. This may explain the decreased escape distance in osmotically stressed smolts attacked from this direction. The reduced escape distance of stressed fish attacked from behind may be explained in terms of fewer wriggling movements from side to side than observed in stressed fish, producing a larger blind zone (Wardle 1993) in the rear and leading to later predator detection. The greater escape distance of schooling smolts than single smolts might also be due to a greater detection distance.

The changes in antipredator behaviour associated with osmoregulatory problems are generally assumed to be adaptive, although they are correlated with greater mortality. The balance between the cost of staying and the cost of fleeing could determine the response to a predator (Ydenberg and Dill 1986). Escape distance ought to decrease in osmotically stressed fish, as too-frequent reactions to predators might result in increased lactate accumulation in muscle, with negative consequences for later attacks and the osmoregulatory capacity. It is assumed that the capacity to adapt to osmotic changes varies from fish to fish, and that single smolts have more serious problems than schooling smolts. When osmoregulatory problems increase during seawater acclimation, the cost of schooling, i.e., constant swimming activity, could reach a critical level that outweighs the benefits, i.e., increased vigilance (Pulliam 1973; Magurran et al. 1985) and dilution effects (Morgan and Godin 1985). At this level, a fish that cannot handle the osmotic stress should leave the school. Swimming alone would then make the best of a bad situation.

In nature the high rate of predation mortality on Atlantic salmon during seaward migration has been explained by the accumulation of predators such as cod in estuaries and adjacent areas (Hvidsten and Møkkelgjerd 1987), and controlled experiments have shown that the presence of predators results in a cumulative stress response (Järvi 1989*a*) and influences predator avoidance (Järvi 1990). Our results show a correlation between physiological changes, changes in antipredator behaviour, and mortality rate. Even though this study was done under controlled conditions, with no cover for the smolts, the results show that there is an interaction between physiological and behavioural mechanisms that should be similar to those occurring in naturally migrating smolts. In the River Imsa tracking studies on ultrasonically tagged migrating smolts have shown that the fish leave the estuary within a few hours (Holm et al. 1982; Jonsson et al. 1993) to be exposed to full-strength seawater and marine predators. This demonstrates that wild smolts too are subjected to a change in salinity during a short period of time.

In conclusion, if osmotic stress had no effect on the ability of a smolt to avoid predation, the probability of being captured ought to have been independent of the length of time the smolts had spent in seawater. The fourfold increase in mortality rate for smolts exposed for 12 and 24 h, i.e., the same period of exposure as that at which the chloride ion and hematocrit levels were found to be highest, suggests that osmotic stress, with a resulting change in antipredator behaviour, explains the increase in mortality rate observed in our experiments. Our results may thus explain some of the heavy smolt losses reported in the River Imsa (Hansen 1981) and other areas in Norway (Hvidsten and Møkkelgjerd 1987; Hvidsten and Lund 1988).

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This explanation is supported by the fact that smolt predation is less severe in brackish waters, such as the Baltic, than in seawater (for review see Bley and Moring 1988; Isaksson 1988; Larsson 1985).

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Comparison of commercial fishery and research catch data

David S. Fox and Richard M. Starr

Abstract: Participants in the U.S. west coast groundfish trawl fishery are required to maintain logbook records that provide a detailed set of species, catch, and effort data with broad temporal and spatial coverage. We used a geographic information system to compare catch data collected from the Oregon commercial trawl fishery with data from U.S. National Marine Fisheries Service research cruises conducted at the same time in the same area. We compared catch locations, catch rates, and biomass estimates for five species, for 1980–1989. Patterns of distribution and relative abundance derived from logbook data closely resembled those from National Marine Fisheries Service triennial trawl research data. Summer season density estimates from logbook data displayed narrower 95% confidence intervals than estimates derived from an entire year, suggesting that sampling in summer months maximizes the statistical reliability of research results. Graphical and statistical analyses indicated that information derived from logbooks can augment research data and improve estimates of the distribution and relative abundance of commercial fish species.

Résumé : Les participants à la pêche du poisson de fond au chalut sur la côte ouest des États-Unis doivent tenir des journaux de bord qui fournissent une série détaillée de données sur les espèces, les captures et l'effort, avec une vaste couverture spatiale et temporelle. Nous avons utilisé un système d'information géographique pour comparer les données sur les captures recueillies par le secteur de la pêche commerciale au chalut en Oregon aux données obtenues par les campagnes de recherche menées en même temps et dans la même région par le U.S. National Marine Fisheries Service. Nous avons comparé la localisation des captures, les taux de capture et les estimations de la biomasse pour cinq espèces, sur la période 1980–1989. Les patrons de distribution et d'abondance relative tirés des données des journaux de bord ressemblaient étroitement aux données des campagnes triennales de recherche au chalut menées par le National Marine Fisheries Service. Les estimations de la densité pendant la période estivale établies à partir des données des journaux de bord présentaient des intervalles de confiance plus étroits à 95% que les estimations tirées de l'année entière, ce qui permet de penser que l'échantillonnage pratiqué pendant les mois d'été maximise la fiabilité statistique des résultats de la recherche. Les analyses graphiques et statistiques indiquent que l'information tirée des journaux de bord peut enrichir les données scientifiques et améliorer les estimations de la distribution et de l'abondance relative des espèces de poissons commercialement exploitées.
[Traduit par la Rédaction]

Introduction

The west coast of the United States is home to a well-developed commercial trawl fishery. Over 377 vessels operated in the Oregon, Washington, and California groundfish trawl fishery in 1991; the reported total catch was more than 278 000 t (Pacific Fishery Management Council 1991). The west coast states, National Marine Fisheries Service (NMFS), and Pacific Fishery Management Council (PFMC) jointly manage catch and effort in the groundfish fishery using a variety of methods such as limited entry, gear restrictions, catch quotas, and trip limits. In a review of PFMC stock assessment methodologies, a U.S. National Academy of Sciences panel suggested that the PFMC consider the use of fishery logbooks to augment research data (Pacific Fishery Management Council 1995).

Oregon trawlers have been required to maintain fishery records since the 1960s, and the PFMC has required fishers to keep records since 1984. These fishery logbooks provide a detailed set of catch and effort data with broad temporal and spatial coverage. In addition to logbook records, an elaborate coastwide monitoring system provides records of the actual weights of fish sold to processors (Pacific Fishery Management Council 1991). Although large amounts of site-specific information are compiled as part of these coastwide data collection programs, much of the data are not used for assessing fish populations, primarily because of the large volume of data and concerns about potential data biases.

Fishery logbooks were used by Gabriel and Tyler (1980), Gabriel (1982), and Tyler et al. (1984) to describe the distribution of commercial fishing effort off Oregon and Washington. Until recently, however, coastwide catch information has been collected, but typically not analyzed, partially because of the complexity of managing and plotting large numbers of geographic data points. With the availability of efficient geographic information systems (GIS), tools now are available to display and analyze large spatial data bases.

We adapted and used a GIS to compare data from the Oregon commercial trawl fishery with data from NMFS research cruises conducted at the same time in the same area (Fig. 1). Our objectives were to map fishery and research catch locations for five species and compare catch distribution patterns, catch rates, and biomass estimates. We suggest that if logbook

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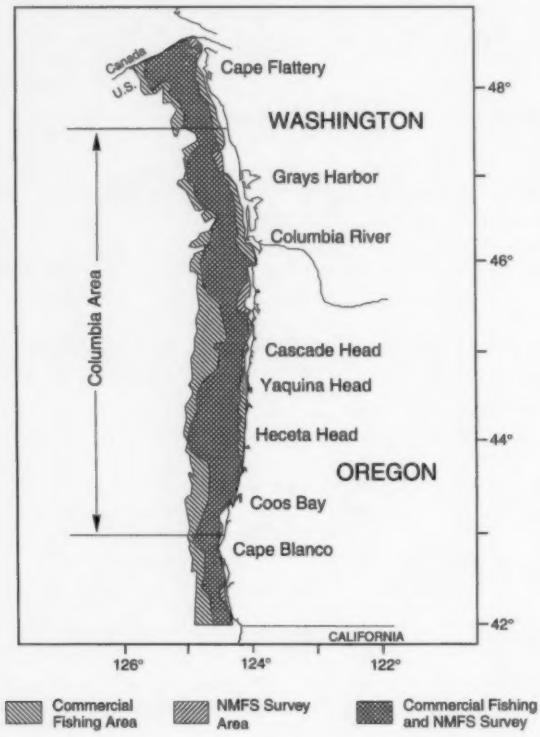
Fig. 1. Commercial fishing and NMFS survey areas in the Columbia Fishery Management Area along the Oregon and Washington coast.



and research data are closely correlated, then logbook data can complement research data and can be used to improve estimates of the distribution and abundance of selected species.

Methods

Data sets

Commercial fishing data used in these analyses were compiled from groundfish bottom trawl fishery logbooks collected by the Oregon Department of Fish and Wildlife (ODFW) from 1980 to 1989. This 10-year logbook data base contains over 130 000 individual records, each representing a single trawl tow. Information for each record includes vessel number, gear type, port of landing, date, latitude and longitude, effort in trawl hours, and catch in pounds for each species or market category reported. Three major gear and net configurations are used in the west coast groundfish fishery: midwater trawls, bottom trawls equipped with roller gear, and bottom trawls without roller gear (sole nets). Our analysis included only the two bottom trawl gear configurations. We computed catch per unit effort (CPUE), expressed in kilograms per hour, for each species in each tow. We had insufficient information to adjust effort for differences in fishing power among the vessels, years, or gear configurations. For a similar reason, we did not adjust catch for discard of unwanted fish at sea.

The procedures used for collecting and processing logbook data included catch recording by fishers, logbook collection, logbook screening, computer data entry, and error checking. The numerous fishing vessels and trips provided a large quantity of data, so we were selective of information included in the analyses. Only those logbooks that were complete and legible and had location information for each tow were analyzed. We used only those logbooks that had closely corresponding fish landing records, enabling the use of weights recorded at the dock. Computer error checking procedures included automated and manual review checks to detect tows that had unreasonable locations with respect to depth or distance from a previous tow. The application of these quality assurance steps resulted in the use of about 50% of the tows executed by the fishery.

The scientific fishery data used in this study originated from a series of NMFS Pacific west coast bottom trawl surveys of groundfish resources conducted in 1980, 1983, 1986, and 1989. The surveys, often referred to as triennial trawl surveys, contained excellent spatial and temporal overlap with the commercial fishery data set. NMFS sampled depths ranging from 55 to 366 m. The surveys were conducted from early July to early October of each survey year. NMFS completed tows at 1572 stations during the four surveys in the Oregon–Washington area; the commercial logbook data set contained 13 244 tows for the months and geographic areas that overlapped with the NMFS surveys.

Triennial trawl survey methods are described by Gunderson and Sample (1980), Weinberg et al. (1984, 1994), Coleman (1986, 1988), Dark and Wilkins (1994), and Weinberg (1994). NMFS used a Nor'eastern high-opening bottom trawl equipped with roller gear, which was similar in size and construction to most commercial roller trawl gear used during the 1980s, except for the cod-end mesh size. NMFS used a 32-mm cod-end mesh liner, whereas the commercial fleet generally used a cod-end mesh of between 76 and 114 mm. NMFS trawled at a speed similar to that of commercial bottom trawlers, but tows were about 0.5 h long, whereas commercial bottom tows averaged 2.8 h.

Our analysis included five species: Dover sole (*Microstomus pacificus*), English sole (*Pleuronectes vetulus*), sablefish (*Anoplopoma fimbria*), yellowtail rockfish (*Sebastes flavidus*), and shortspine thornyhead (*Sebastolobus alascanus*). Dover sole, English sole, and sablefish were reported in logbooks as individual species throughout the 1980s. Yellowtail rockfish has been reported by trawlers separately since 1985 and thornyhead has been reported since 1984 as a combination of shortspine and longspine thornyhead (*Sebastolobus altivelis*). ODFW catch sampling conducted in 1989, however, indicated that the commercial catch of thornyhead consisted of at least 92% shortspine in the areas and times that coincided with the NMFS surveys (Oregon Department of Fish and Wildlife, unpublished data).

We examined the relative contribution of fishes in the NMFS catch that were below the size that would be commercially retained but we did not have size-frequency information for each tow and thus could not adjust NMFS catches to account for submarket-sized fishes. The size thresholds for commercial retention of the five study species were determined from ODFW market sample data (Oregon Department of Fish and Wildlife, unpublished data).

We used three approaches to compare commercial and research catch data. First, we compared spatial patterns of catch distribution by overlapping coastwide isopleths of CPUE. Second, we compared NMFS and logbook CPUE stratified by depth and latitude using statistical and graphical techniques. Finally, we compared species biomass estimates derived with similar methods for each data type.

Spatial patterns of CPUE

We visually compared spatial patterns of CPUE by examining contour maps developed from logbook and research catches. Logbook data used for the contour maps included only those trawls that fell within the same time frame and depth range as each of the NMFS cruises. We used all tows deemed adequate for analysis by NMFS. For both data types, analyses included tows that caught the species of interest as well as tows with no catch of the species.

We computed and plotted isopleths representing the 50th, 75th, and 90th percentile levels of CPUE for each species, year, and data type, using commercial GIS and contouring software packages. The 50th percentiles of CPUE for yellowtail rockfish and shortspine

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thornyhead were either zero or nearly zero because there were many tows with no catch of the species. Therefore, only the 75th and 90th percentile isopleths were mapped for those species.

For each species, we compared logbook and research catch locations by overlaying the CPUE maps and evaluating the geographic co-occurrence of the polygons defined by the isopleths. We also computed the total surface area of polygons and the total area of overlap between the two data types. The amount of spatial overlap was calculated by determining the percentage of the surface area covered by logbook CPUE isopleths that co-occurred with corresponding levels of CPUE from the NMFS data.

Graphical and statistical comparison of CPUE

As a second method of comparing CPUE, we examined NMFS and commercial fishery data graphically and statistically. Both data sets were stratified by year, increments of 94 m of depth (50 fthm), and 30 min of latitude. There were 52 possible strata (4 depth increments by 13 latitude increments) in the area sampled by each of the NMFS surveys. We transformed the individual tow CPUE values using a $\ln(x + 1)$ transformation (Zar 1974), and calculated frequency of occurrence and mean CPUE for both research and logbook data sets. We compared frequency distributions using the Kolmogorov–Smirnov two-sample test (Sokal and Rohlf 1981).

We used a parametric simple linear correlation analysis (Snedecor and Cochran 1967) on the transformed data to compare logbook and NMFS mean CPUE. Mean CPUE represents the sum of individual tow CPUE divided by the number of tows. The computations included both tows that caught the target species and those with no catch. We computed separate mean CPUE values for roller net, sole net, and combined bottom nets to analyze the effects of different net types on the correlation analysis.

In the logbook data set, we calculated mean CPUE for all strata that contained 10 or more tows; sample size ranged from 10 to 1180 tows per stratum. We used a threshold of 10 tows to minimize spurious CPUE values resulting from data collected from a single vessel or fishing trip. The computation of mean CPUE in the research data set included all tows within each stratum that were deemed adequate for analysis by NMFS (National Marine Fisheries Service 1989). Research sample size ranged from 1 to 120 tows per stratum.

Biomass comparisons

Our third approach to data comparison involved estimating biomass from the logbook data and comparing the estimates with similar values generated from NMFS data. We estimated biomass from the logbook data using an area swept methodology similar to that employed by NMFS (Gunderson and Sample 1980). We obtained footrope specifications and estimates of net opening width for several gear types used in Oregon (Wathne 1977; Amos 1985; Danish Institute of Fisheries Technology 1987). On the basis of information from 13 types of trawl nets, opening width averaged 41.6% (range 29.0–49.0%) of the footrope length. Footrope length of bottom trawl gear averaged 22.8 m, with a range of 17.2–39.1 m (Oregon Department of Fish and Wildlife, unpublished data from a 1985 and 1986 survey of the Oregon trawl fleet). Applying the average ratio of net opening width to average footrope length, we estimated an average net opening width for the trawl fleet of 9.5 m. For comparison, NMFS used a trawl net with a 32.2-m footrope and an opening width of 42% (13.5 m) (Gunderson and Sample 1980). Commercial vessel speed averaged 4.5 km/h with a range of 3.3–5.6 km/h (Oregon Department of Fish and Wildlife, unpublished data from a 1985 and 1986 survey of the Oregon trawl fleet). Prescribed trawling speed for triennial trawl surveys was 5.6 km/h.

We calculated the average area swept per trawl hour for commercial gear to be 4.2 ha/h. A similar computation for NMFS gear yielded a value of 7.5 ha/h. Employing the same data stratification scheme of depth and latitude described above, we estimated surface area with the GIS software, and then estimated biomass for each stratum using logbook data as follows:

$$\text{biomass within each stratum (kg)} = (\text{stratum area (ha)} / 4.2\ \text{ha/h}) \times \text{mean stratum CPUE (kg/h)}$$

We produced two types of biomass estimates from fishery logbook data. The first method provided a biomass estimate that was comparable with NMFS triennial trawl survey biomass estimates for the Columbia Fishery Management Area. We computed a biomass for each stratum using only July–September data and summed only the strata within the Columbia Fishery Management Area (43°00′ to 47°30′) and three depth increments from 94 to 360 m. To make the NMFS and logbook data directly comparable, we divided biomass by total strata surface area to produce a catch weight per hectare. We computed 95% confidence intervals (CI) for each estimate using methods described in Cochran (1977). NMFS computed 95% CI using the same methods (Gunderson and Sample 1980).

For the second estimate of biomass from logbooks, we computed an average annual CPUE per stratum by first calculating four seasonal CPUE values and then averaging the seasonal values within each stratum. We then summed the strata within the Columbia Fishery Management Area between 94 and 360 m and divided the resultant biomass estimates by the total surface area of the strata. These biomass estimates allowed us to explore the effects of sampling during one season relative to year-round sampling.

Table 1. Percentiles of CPUE (kg/h) calculated from commercial logbook and NMFS triennial trawl cruise data.

		Logbook CPUE percentiles			NMFS CPUE percentiles		
	Year	50th	75th	90th	50th	75th	90th
Dover sole	1980	88.0	129.3	159.7	7.5	27.9	60.7
	1983	49.6	97.7	153.6	14.5	55.5	104.7
	1986	38.4	87.8	157.4	18.6	43.5	89.9
	1989	54.4	102.0	143.7	17.0	37.1	75.5
Sablefish	1980	4.8	33.5	105.9	4.0	18.5	160.6
	1983	9.3	36.5	61.1	8.1	35.3	119.5
	1986	7.8	30.3	70.0	11.8	41.8	90.7
	1989	8.8	19.5	39.0	3.4	27.1	121.7
English sole	1980	3.1	13.5	27.5	0.5	2.7	19.1
	1983	3.3	9.8	24.5	2.2	11.9	27.6
	1986	1.0	16.0	34.7	2.7	16.1	35.6
	1989	3.4	16.3	37.6	2.7	23.3	49.1
Yellowtail rockfish	1986		0.7	32.7		9.3	55.3
	1989		14.5	93.3		0.9	21.8
Shortspine thornyhead	1986		3.8	10.8		3.2	13.0
	1989		6.2	21.6		3.6	12.3

Results

Spatial comparison

For all species, the 50th, 75th, and 90th percentile levels of CPUE were of similar magnitude (Table 1) and spatially co-occurred (Fig. 2). In each of the data sets, the highest catch rates for Dover sole occurred off Cape Flattery, Gray's Harbor, the Columbia River, Cascade Head, Heceta Head, and Cape Blanco (Fig. 2). Centers of the 90th percentile of research and logbook CPUE were in similar locations from year to year. Isopleth maps of 1980 data showed areas of high research

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Fig. 2. Isopleths of the 50th, 75th, and 90th percentiles of CPUE for commercial fishery and NMFS triennial trawl cruises for Dover sole, sablefish, English sole, yellowtail rockfish, and shortspine thornyhead.

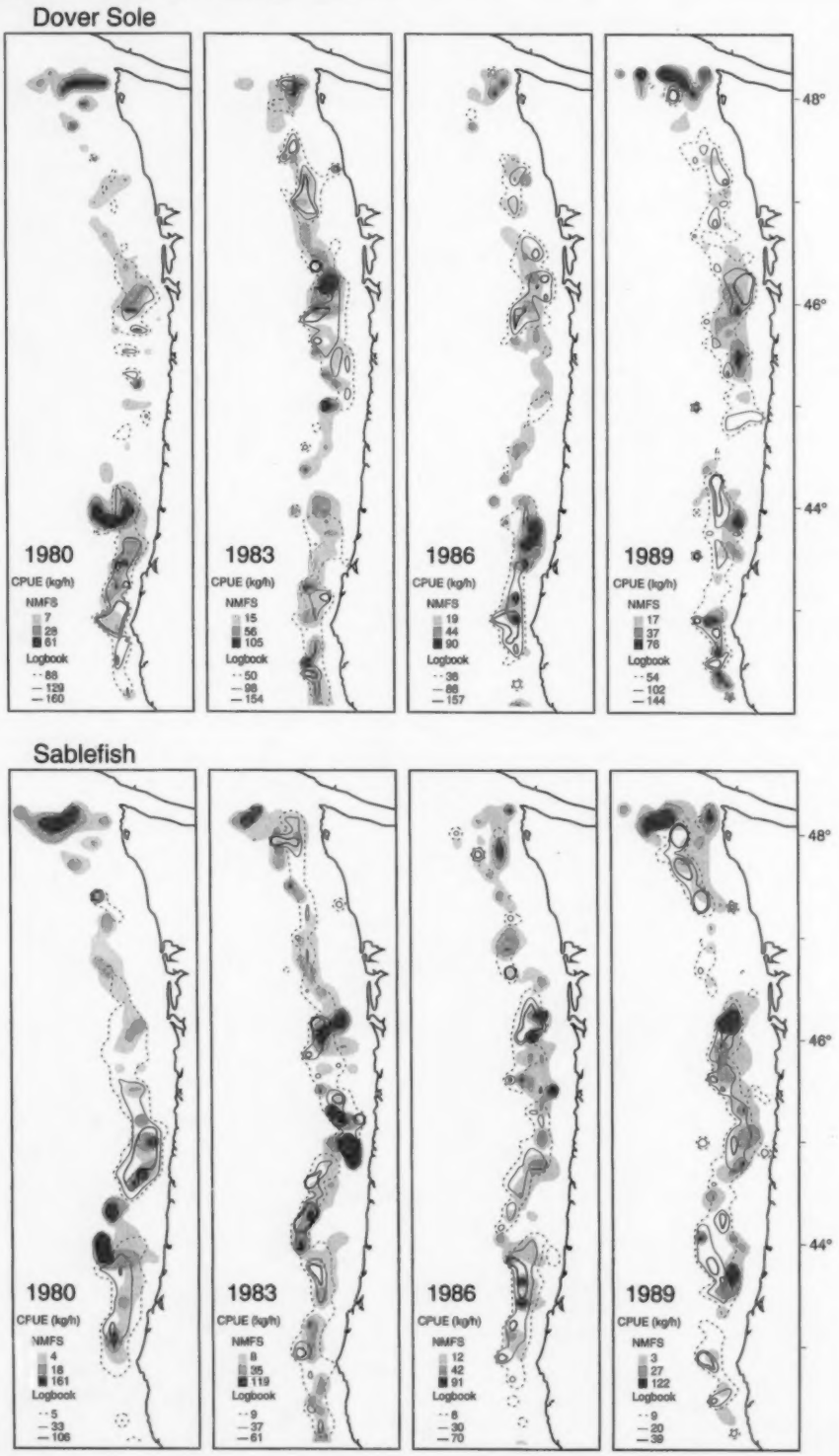

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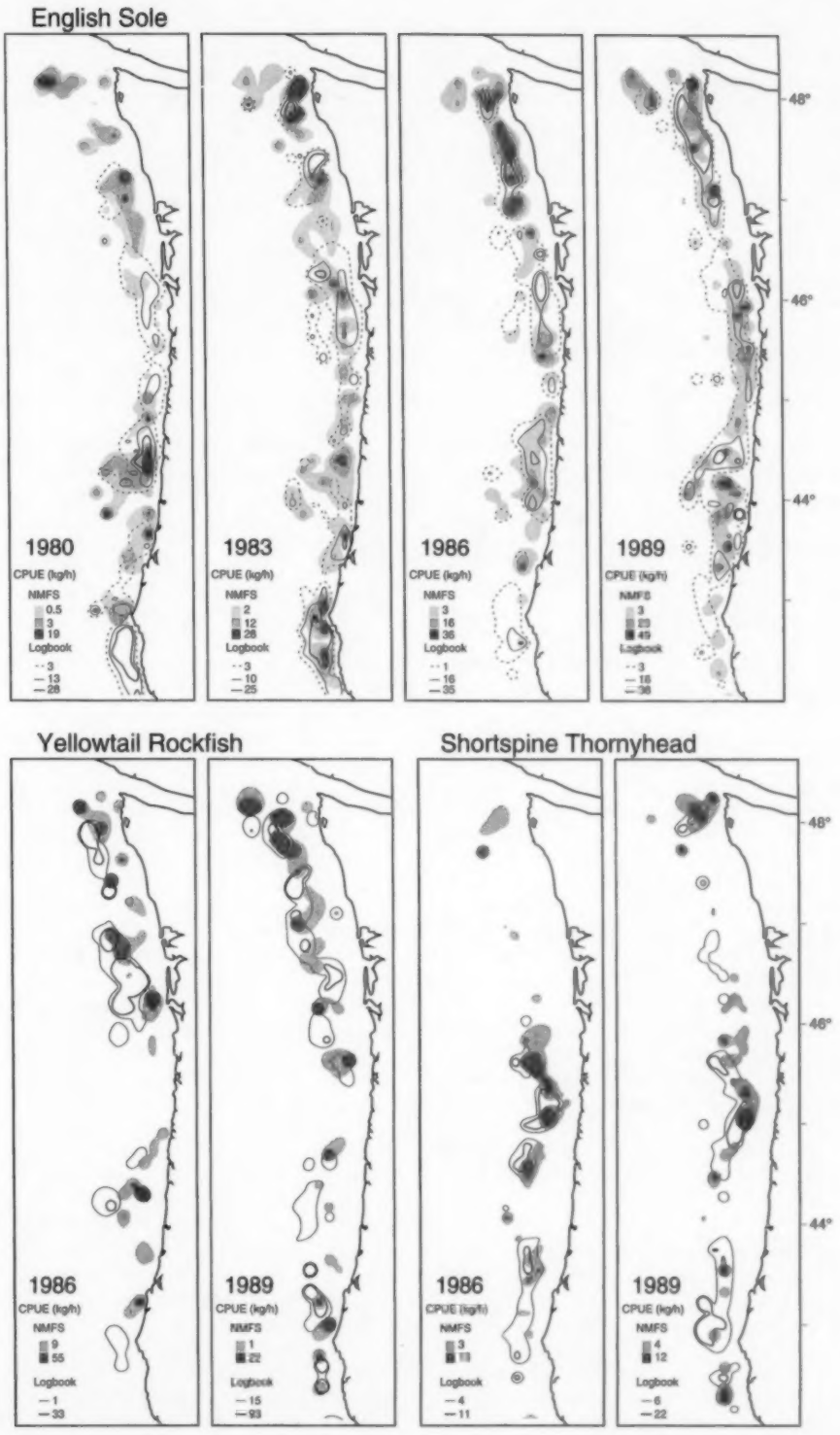
Fig. 2 (*concluded*).



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CPUE off Cape Flattery without corresponding high logbook catch rates. Conversely, logbook data showed high catch rates off southern Oregon in 1980 without corresponding high research catch rates. In each case, the discrepancy was caused by lack of either commercial or research sampling effort (Fig. 3).

The highest catch rates for sablefish occurred off Cape Flattery, the Columbia River, and Cascade Head, and from Heceta Head to Cape Blanco (Fig. 2). Centers of the 90th percentile of research and logbook CPUE were in similar locations with two exceptions. NMFS cruises in 1980 and 1983 showed high catch rates off Cape Flattery that were not apparent from logbook data. In both cases, there was a lack of commercial fishing effort in those areas (Fig. 3).

The highest catch rates for English sole occurred off northern Washington, Gray's Harbor, the Columbia River, Yaquina Head, Heceta Head, and southern Oregon (Fig. 2). Centers of the 90th percentile of research and logbook CPUE were in similar locations, except that CPUE maps for 1980 and 1986 showed generally lower research catch rates off the Columbia River. Logbook catch rates for 1980 and 1986 in southern Oregon also were high without corresponding high research catch rates, but those differences can be attributed to a lack of research sampling effort (Fig. 3).

The highest catch rates for yellowtail rockfish occurred off northern Washington, Gray's Harbor, Cascade Head, Heceta Head, and Cape Blanco. Centers of the 90th percentile of research and logbook CPUE were in similar locations, but logbook data indicated larger areas of high catch rates than did the research data. Similarly, locations of high CPUE for shortspine thornyhead overlapped with triennial trawl data, but logbook data suggested a broader distribution of high CPUE compared with the research data.

In terms of percent overlap between data types, more than 66% of high logbook catch locations (defined by isopleths of 50th percentile of CPUE) occurred in the same area as high research catches for Dover sole, English sole, and sablefish (Table 2). Overlap ranged from 60 to 81% for the 50th percentile polygons, from 23 to 63% for the 75th percentile polygons, and from 13 to 47% for the 90th percentile polygons (Table 2). Overlap of surface area for shortspine thornyhead CPUE isopleths averaged 58 and 57%, respectively, for the 75th and 90th percentiles of CPUE. Overlap of surface area for yellowtail rockfish CPUE isopleths averaged 40 and 19%, respectively, for the 75th and 90th percentiles of CPUE.

English sole and shortspine thornyhead below market size contributed relatively large amounts to NMFS catch (Table 3), but we found no obvious areas where this caused NMFS and logbook data to differ in spatial distribution. For the 1 year in which we had research length-frequency information by tow, concentrations of submarket-sized English sole were caught in areas that also contained high commercial English sole catches.

Graphical and statistical comparison of CPUE

Both research and logbook data bases contained a large proportion of tows with no catch of a species. Even after the data were transformed, the frequency distributions were skewed (Fig. 4). The ranges of CPUE and shapes of the frequency histograms were similar between research and logbook data. The most obvious difference between the two data sets was the occurrence of a larger percentage of smaller catches in the research data set. A Kolmogorov–Smirnov two-sample test showed no significant differences between frequency distributions for any of the species except yellowtail rockfish (Table 4).

Linear correlation analysis comparing logbook and research CPUE yielded significant results in all but one case (Table 5). Dover sole CPUEs were correlated for sole nets ($P < 0.05$) but not for roller nets. Correlation coefficients were similar between gear types for most species. For sablefish, however, the correlation coefficient was almost two times higher for sole nets than for roller nets.

Biomass comparisons

Logbook biomass estimates for all species were similar in magnitude to those derived from research data, with the exception of logbook biomass estimates for Dover sole, which were three to six times greater than those derived from research catches (Fig. 5). Biomass estimates for English sole, sablefish, and yellowtail rockfish exhibited overlapping 95% CI for all years compared.

For Dover sole, logbook biomass estimates exhibited a general decreasing trend from 1980 through 1985. The trend appeared to be level or slightly increasing from 1986 through 1989; a linear regression fitted to all years yielded a line with a negative slope ($P < 0.05$; Table 6). The biomass trend derived from research catches was flat for Dover sole for the entire period. Because much of the commercial fishery for Dover sole occurs beyond the range of the triennial trawl cruises, we compared logbook biomass estimates with NMFS slope survey biomass estimates (Table 7). The 95% CI of the 1984, 1988, and 1989 slope surveys overlapped with the corresponding estimates from the logbook data.

For sablefish, logbook biomass estimates generally decreased, except for slight increases in 1986 and 1987. A linear regression fitted to all years yielded a line with a negative slope (Table 6); the regression was highly significant ($P < 0.001$) when the data were analyzed without the 1987 data. Biomass trends derived from research catches fluctuated out of phase with logbook estimates, and a linear regression fitted to all years yielded a line with a slightly positive slope.

Biomass estimates generated from logbooks for English sole were consistent from 1980 to 1985, then sharply increased from 1985 to 1989. A line fit to all years had a significantly positive slope ($P < 0.05$). Biomass trends derived from research catches were similar.

Biomass estimates generated from logbooks for yellowtail rockfish increased each year from 1985 to 1989. A linear regression fit to all years yielded a line with a positive slope. The two research data points resulted in a level trend in biomass. Logbook biomass estimates for shortspine thornyhead rockfish exhibited a level trend from 1984 to 1988. The biomass estimate for 1989 was twice that of previous estimates. The two research data points resulted in a decreasing trend in biomass.

For each species, biomass estimates derived from commercial fishery logbook data for the summer season were similar to estimates derived for an entire year (Fig. 6). An average of 34% of the fishing effort occurred in the summer. For both Dover sole and sablefish, biomass estimates derived from year-round data were lower than those derived from one season's data, with overlap of the 95% CI in some years. For English sole, the summer period provided higher biomass

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Fig. 3. Locations of commercial tows used in the logbook data base, and NMFS triennial trawl survey tow locations for the years 1980, 1983, 1986, and 1989.

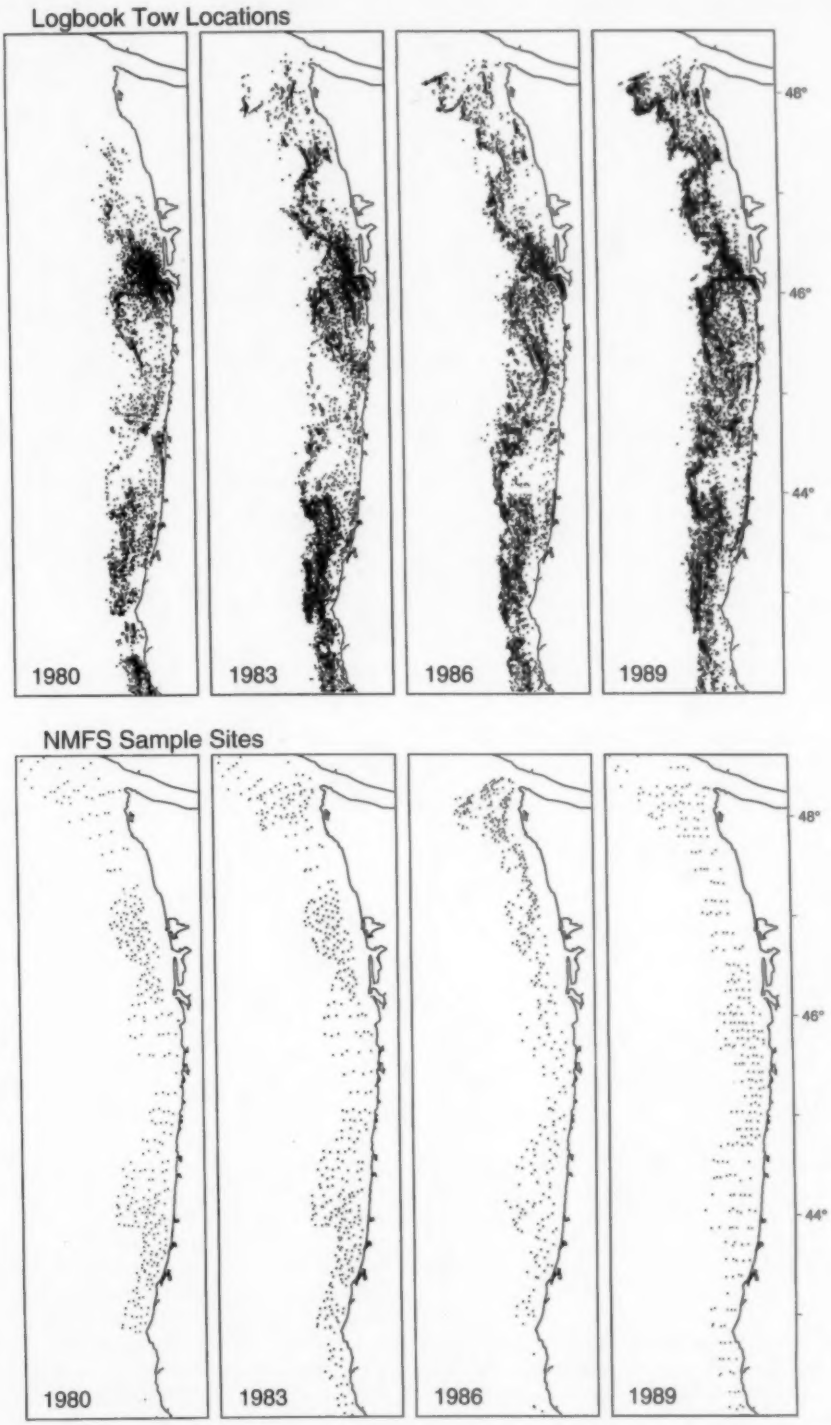

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Table 2. Spatial overlap between NMFS and commercial catch areas.

	Year	Percentiles 50th	75th	90th
Dover sole	1980	60.3	47.1	15.4
	1983	74.4	44.1	25.0
	1986	75.9	41.4	33.3
	1989	60.8	22.6	12.5
	Mean	67.9	38.8	21.6
Sablefish	1980	65.0	44.8	23.1
	1983	80.5	62.9	46.7
	1986	76.1	41.7	44.4
	1989	72.6	51.9	25.0
	Mean	73.6	50.3	34.8
English sole	1980	66.7	54.5	40.0
	1983	65.0	47.2	33.3
	1986	66.7	48.3	30.8
	1989	66.7	50.0	22.7
	Mean	66.3	50.0	31.7
Yellowtail rockfish	1986		41.4	16.7
	1989		38.5	21.4
	Mean		40.0	19.1
Shortspine thornyhead	1986		64.7	80.0
	1989		51.7	33.3
	Mean		58.2	56.7

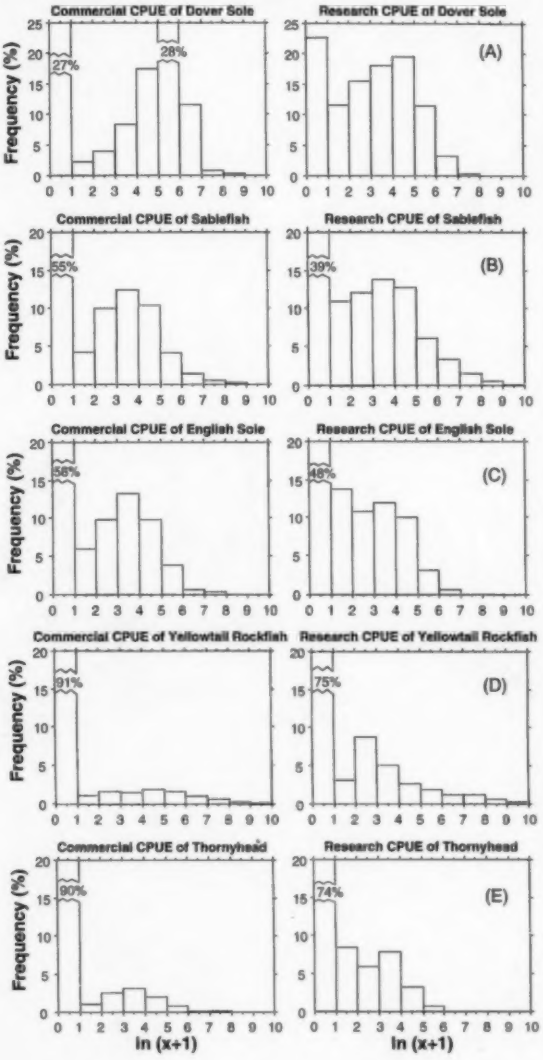
Note: Values are percent overlap of surface areas enclosed by the 50th, 75th, and 90th percentile CPUE isopleths of each data type. Data reflect only areas that included sampling effort for both NMFS and logbook data sets.

Table 3. Estimated minimum market size of selected species caught in the commercial fishery and percentage of NMFS survey catch (by weight) that was below market size.

	Estimated minimum market size (cm)	% NMFS catch less than minimum market size
Dover sole	30	15.5
Sablefish	41	11.9
English sole	30	48.7
Yellowtail rockfish	36	8.8
Shortspine thornyhead	27	61.0

Note: Percentages are average estimated values from the 1980, 1983, 1986, and 1989 triennial trawl surveys. Minimum market sizes were derived from ODFW port sampling data (Oregon Department of Fish and Wildlife, unpublished data).

Fig. 4. Frequency histograms of $\ln(x + 1)$ transformed CPUE values derived from commercial fishery logbooks and NMFS triennial trawl cruises for (A) Dover sole, (B) sablefish, (C) English sole, (D) yellowtail rockfish, and (E) shortspine thornyhead.



estimates from 1980 to 1982. The 95% CI for all other years overlapped between seasons. For yellowtail rockfish and shortspine thornyhead, the 95% CI overlapped between seasons for all years.

Discussion

Comparison of logbook and research catch data

West coast fishery logbook data receive only limited use in stock assessments because scientists and managers have assumed that logbook CPUE data do not provide an accurate index of fish abundance (Ianelli et al. 1994; Methot et al. 1994; Turnock et al. 1994). This assumption is based on the concept that research catches are more apt to reflect actual fish distribution and abundance, whereas commercial fishing patterns and catches are more greatly influenced by market conditions, regulations, weather, and proximity to port. Although recent comparisons of trawl, submersible, and remotely operated vehicle surveys suggest that trawl sampling may not accurately reflect the distribution and abundance of many species (Kulbicki and Wantiez 1990; Krieger 1993; Adams et al. 1995), we chose to use the triennial trawl cruise data as a reference with which to compare commercial catches. These two data sets represent catches from similar fishing gear and methods. If fish distribution and abundance were not important determinants of commercial fish catches, we would have expected logbook

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Table 4. Results of Kolmogorov–Smirnov two-sample comparison of frequency distributions of ln(*x* + 1) transformed CPUE calculated from commercial and research catches.

	df	No. of intervals	Maximum difference	χ^2	*P*
Dover sole	2	10	0.60	7.20	0.055
Sablefish	2	10	0.60	7.20	0.055
English sole	2	10	0.50	5.00	0.164
Yellowtail rockfish	2	10	0.70	9.80	0.015*
Shortspine thornyhead	2	10	0.50	5.00	0.164

Note: *, $P < 0.05$.

catch patterns to greatly deviate from research catch patterns. However, the logbook and research data sets clearly showed the same specific areas that consistently produced high catch rates, and logbook and research catch rates were significantly correlated.

There were a few time–area–species combinations in which logbook and NMFS CPUE isopleths did not coincide. Many of the areas of poor spatial correspondence can be explained by the lack of research or commercial trawl effort in the area. Some of the areas of poor spatial correspondence between the logbook and NMFS data cannot be easily explained and may reflect actual differences between research and commercial fishing techniques or gear or may be due to the grouping of many different fishing vessels and gear types in the analysis.

The similarity in frequency distribution of CPUE between logbook and research data sets suggests that, overall, the commercial fleet sampled the study area in a similar fashion as did research vessels. The high percentage of zero catches indicates that the species we studied were contagiously distributed and dispersed. Except for Dover sole, the commercial fleet did not appear to have more success targeting fishes than did research vessels. The larger proportion of high catch rates in the commercial fishery for Dover sole probably reflects the differences in gear and fishing techniques between commercial and research operations. Turnock et al. (1994) reported that the roller gear used on NMFS triennial trawl surveys proved to be less efficient for catching Dover sole than standard commercial gear.

In most widely spread fisheries, fishing effort is not evenly distributed, and there is no simple relationship between CPUE and overall fish abundance (Beverton and Holt 1957). Bannerot and Austin (1983) tested the sensitivity of CPUE frequency distribution to changing abundances for a sport fishery and suggested that for many schooling fishes, the relative frequency of zero CPUE values is a better index of abundance than is the mean CPUE. Their technique, however, requires independent estimates of CPUE and abundance. Although the relative frequency of zero CPUE values may provide additional information from research cruises, it is probably not as useful a tool for the commercial fishery, given the high level of fish-finding technology currently in the fleet. Bannerot and Austin (1983) also reported that median CPUE was a better indicator of changes in abundance than mean CPUE when using pooled data. The reverse was true for unpooled data. We used unpooled data because of the importance of maintaining a large sample size (Stanley 1992) and thus used mean CPUE in our statistical comparisons.

Differences in CPUE may also be attributable to a broader size range of fishes retained by the smaller cod-end mesh in research gear. NMFS estimated that Dover sole, sablefish, and yellowtail rockfish below commercial size contributed only a small amount to the triennial trawl survey catches by weight (Table 3) and thus had a minimal effect on overall research CPUE. However, when small fish were distributed in concentrated patches, the catch rates for the strata containing those patches may have been elevated relative to commercial catches.

Table 5. Results of correlation analysis (*r* values) between CPUE calculated from commercial and research catches.

	Bottom net types		
	All nets	Sole net	Roller net
Dover sole	0.42*	0.54*	0.21
	(147)	(82)	(36)
Sablefish	0.68*	0.70*	0.37*
	(148)	(82)	(38)
English sole	0.65*	0.65*	0.52**
	(143)	(83)	(34)
Yellowtail rockfish	0.46*	0.37**	0.51**
	(76)	(67)	(34)
Shortspine thornyhead	0.61*	0.58*	0.33*
	(77)	(68)	(35)

Note: The CPUE data used in the analysis were transformed (ln(*x* + 1)) mean values within strata defined by year and increments of 94 m depth and 30 min of latitude. Numbers in parentheses indicate sample size (numbers of strata with both commercial and research catches). *, $P < 0.05$; **, $P < 0.01$.

The relationship between research and logbook biomass estimates varied from good to poor, depending on species. English sole provided the closest agreement between research and commercial catches, probably because research and commercial fishing techniques were similar and the shallow benthic habitats occupied by English sole were well sampled by both research and commercial trawls.

Dover sole biomass estimates exhibited the greatest difference between research and logbook data. Both data sets displayed relatively low variability, but annual biomass estimates were significantly different ($P < 0.05$). Logbook biomass estimates for Dover sole were 4.7 times greater on average than estimates from triennial trawl surveys. This discrepancy can be explained primarily by differences in fishing gear. Turnock et al. (1994) reported that NMFS slope surveys using chain disk gear caught Dover sole at about four times the rate of the triennial trawl survey gear. Adjusting triennial trawl catches upwards by a factor of four would result in a better correspondence between logbook and triennial trawl survey data. We considered comparing logbook and NMFS slope surveys but decided that a more detailed consideration of the fishing gear used in deeper water would be required to enable a quantitative comparison between logbook and NMFS slope survey data.

Both research and logbook estimates of biomass for schooling yellowtail rockfish were highly variable. Weinberg et al. (1994) described unsuccessful efforts to reduce sample variability for yellowtail rockfish in NMFS surveys by increasing sampling effort. In the much larger logbook data set, the combination of a few extremely high catch rates with incidental catches from many tows also resulted in high variances of yellowtail rockfish biomass estimates. Additional variability was introduced into the logbook data by the fact that the net

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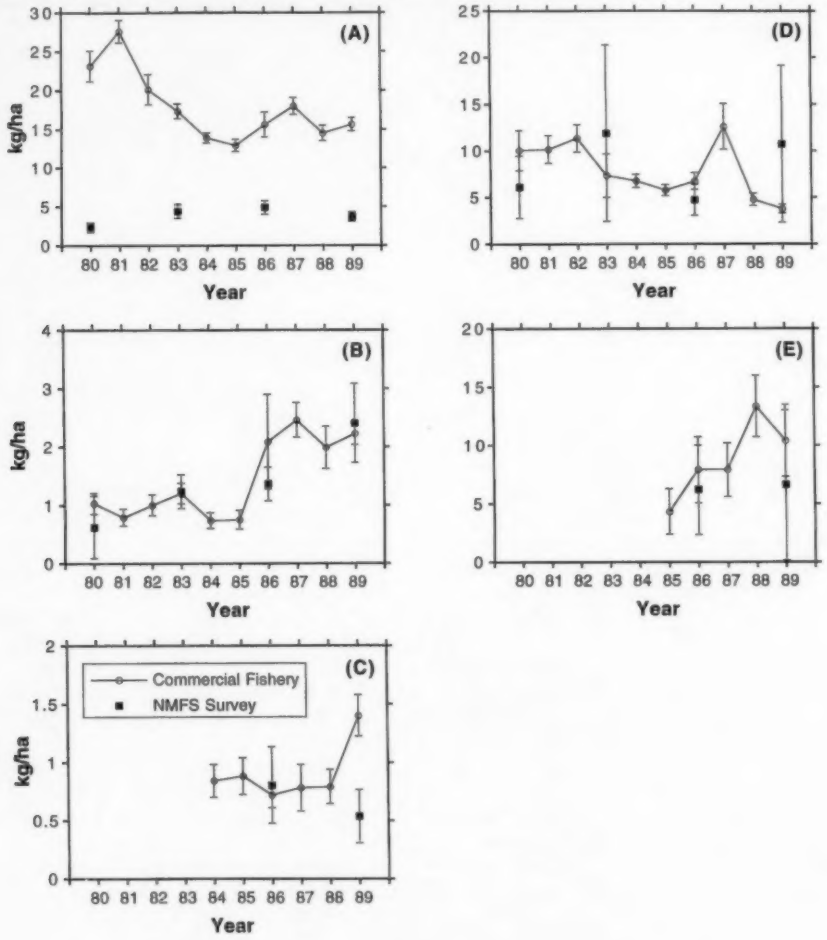
Fig. 5. Biomass estimates and 95% CI derived from trawls conducted from July to September in the commercial fishery and NMFS triennial trawl cruises for (A) Dover sole, (B) English sole, (C) shortspine thornyhead, (D) sablefish, and (E) yellowtail rockfish.



height of different gear types was not considered in the area-swept analysis. An alternative expansion method of partitioning out the small areas occupied by schools (on the basis of high logbook CPUE) from the larger low density areas may provide a less variable CPUE estimate. Although there may be ways to manipulate the data to reduce variability of biomass estimates, Krieger (1993) and Adams et al. (1995) cautioned that trawl surveys poorly estimate the abundance of midwater rockfishes. Richards and Schnute (1986) also suggested that CPUE may be a poor index of abundance for rockfishes.

Logbook and research estimates of biomass for shortspine thornyhead were low, perhaps because of the fact that most individuals reside in the deeper portions of the study area. Since we chose only areas that had both adequate logbook and adequate NMFS tows, the deeper portions of the study area are probably underrepresented.

Use of logbook data to augment research cruises

Our results indicate that data collected from research cruises and the commercial fishery are similar. It follows, then, that commercial fishery logbook data may be used to evaluate or augment research data, especially if the PFMC chooses to manage fisheries in small area units. Logbooks may become the only cost-effective way to increase the amount of information available in specific areas. Since the commercial fishery samples every month of every year and at many thousand sites, logbook data may provide information to help identify sampling error, increase sample size, or fill spatial and temporal gaps in research data. Although logbook data can be used effectively for area management, Stanley (1992) cautions against selecting management areas that are too small to provide sufficient data for adequate estimates of CPUE.

The larger number of biomass estimates generated by logbook data provided a different view of biomass trends for several of the species. The additional information provided by logbooks should be useful in tuning population models and helping managers to identify the degree of risk and uncertainty in stock assessments, thus aiding in the development of

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Table 6. Trends in biomass derived from linear regression analysis applied to annual biomass estimates from logbook and research catch data.

	Slope	Intercept	r	P	MS	F
Dover sole						
NMFS	0.157	−307.1	0.54	0.46	1.1	1.8
Logbook	−1.066	2133.4	0.70	0.02*	93.8	7.9
Sablefish						
NMFS	0.221	−430.9	0.25	0.75	2.2	0.1
Logbook	−0.552	1102.5	0.56	0.09	25.1	3.7
Logbook[a]	−0.775	1545.5	0.91	0.0006***	45.4	35.7
English sole						
NMFS	0.182	−360.5	0.95	0.04*	1.5	20.4
Logbook	0.176	−348.4	0.78	0.007*	2.6	12.7
Yellowtail rockfish						
NMFS	0.133	−258.6	na	na	na	na
Logbook	1.762	−3491.7	0.83	0.08	31.0	6.7
Shortspine thornyhead						
NMFS	−0.076	151.2	na	na	na	na
Logbook	0.074	−145.7	0.55	0.26	0.1	1.8

Note: na, not applicable (line had only two points).*, $P < 0.05$; ***, $P < 0.001$.

[a]Sablefish biomass trends derived from logbook data that excluded an anomalously high biomass estimate in 1987.

risk-based fishery models, such as proposed by Hilborn et al. (1993).

NMFS triennial trawl cruises occur primarily in summer months, whereas the commercial fishery occurs the entire year. We analyzed the logbook data by season to determine if a seasonal estimate of biomass provided a different picture of biomass trends relative to sampling conducted over all seasons. Analysis of the summer logbook data provided as good an indication of interannual trends in biomass as did the analysis of the entire year. Interestingly, although the summer season contained only one third of the total annual sampling effort, biomass estimates displayed consistently narrower 95% CI than estimates derived from an entire year of logbook data. This implies that in addition to the obvious logistical advantages to surveying in the summer, NMFS is maximizing the statistical reliability of the triennial trawl cruise data by sampling in the summer. These results were somewhat different from those presented by Smith (1980) and Large (1992), who demonstrated significant seasonal effects on CPUE in trawl fisheries.

Factors affecting the use of commercial fishery data

Cooperation from a large number of commercial fishers is obviously critical for logbooks to be useful. Proper collection, preparation, and analysis of logbook data are also essential to maximizing the usefulness of the information. Logbook data need to be thoroughly error checked with verification of depth and locational information. Species and pounds hailed on the logbooks need to match the delivery tickets or be close enough to enable correction and adjustment.

Evaluating, then adjusting, estimated catch can create several problems. We created a ratio of estimated catch of a species by tow for each trip, then replaced estimated catch with the product of the ratio times the official landing record. In some instances, a species was caught in small quantities but not recorded in the logbook. In those cases, the total official weight of that species was distributed among all tows. At other times, a species was caught and recorded in large quantities on one tow and caught in small quantities, but not recorded, on other tows. In those cases, all of the official catch of that species was allocated to one tow. In the first instance, the adjustment process would overestimate distribution and underestimate the number of zero catches. In the second instance, the adjustment process would result in a restricted distribution and overestimate the number of zero catches. Kimura (1981) and Stanley (1992) avoided these problems by analyzing only those catch records that contained more than 25% of a species. Although their approach resolves the problem of falsely distributing small catches, Kimura (1981) stated that the use of qualifying criteria can greatly affect estimates of relative abundance. We minimized potential distributional problems by plotting the isopleths representing catches greater than the 50th percentile of CPUE and minimized the effect on mean CPUE by using only logbooks with closely corresponding landing records.

Table 7. Biomass estimates and 95% confidence intervals (CI) derived from the 1984, 1988, and 1989 NMFS slope surveys and commercial data from the same depths and latitudes as the corresponding slope surveys.

	Slope surveys		Logbook data	
Year	Biomass (kg/ha)	95% CI (kg/ha)	Biomass (kg/ha)	95% CI (kg/ha)
1984	20.0	5.6	19.2	4.8
1988	41.2	28.7	25.5	9.4
1989	45.8	11.5	24.6	7.6

Note: Summaries of the slope surveys were obtained from Raymore and Weinberg (1990) and Parks et al. (1993).

Market demand, quotas, and by-catch regulations all influence fishing operations and thus the landed catch of vessels. Occasionally market demand is large enough to start a new fishery or completely stop catch of a species for a period. The effects of usual market demands, however, are difficult to distinguish from other factors affecting fishing strategies. We made no attempt to account for market influences on fishing patterns; we were primarily interested in distributional patterns of reported catch.

Sampson (1991) modeled factors that lead commercial fishers to choose a fishing location and concluded that distance from port can be a major factor influencing the selection of a fishing ground. In his model, distance from port affected CPUE, and he cautioned against calculating CPUE using a simple ratio of total catch to total effort. We agree that CPUE should not be calculated as a simple ratio and agree with Stanley (1992) that it is important to carefully select the size of the spatiotemporal unit used to represent a central tendency of CPUE. Using individual tows rather than pooled data provided a large enough sample size to enable us to estimate CPUE in small time and area units.

The discard of fishes in the commercial fishery provides a potentially major discrepancy between logbook and research estimates of fish abundance. The problem is especially difficult to address if discard occurs at different rates in different locations. If discard rates are known and are consistent in time and space, however, biomass estimates derived from logbooks

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Fig. 6. Biomass estimates and 95% CI derived from trawls conducted from July to September in the commercial fishery compared with biomass estimates derived for an entire year from the commercial fishery for (A) Dover sole, (B) English sole, (C) shortspine thornyhead, (D) sablefish, and (E) yellowtail rockfish.

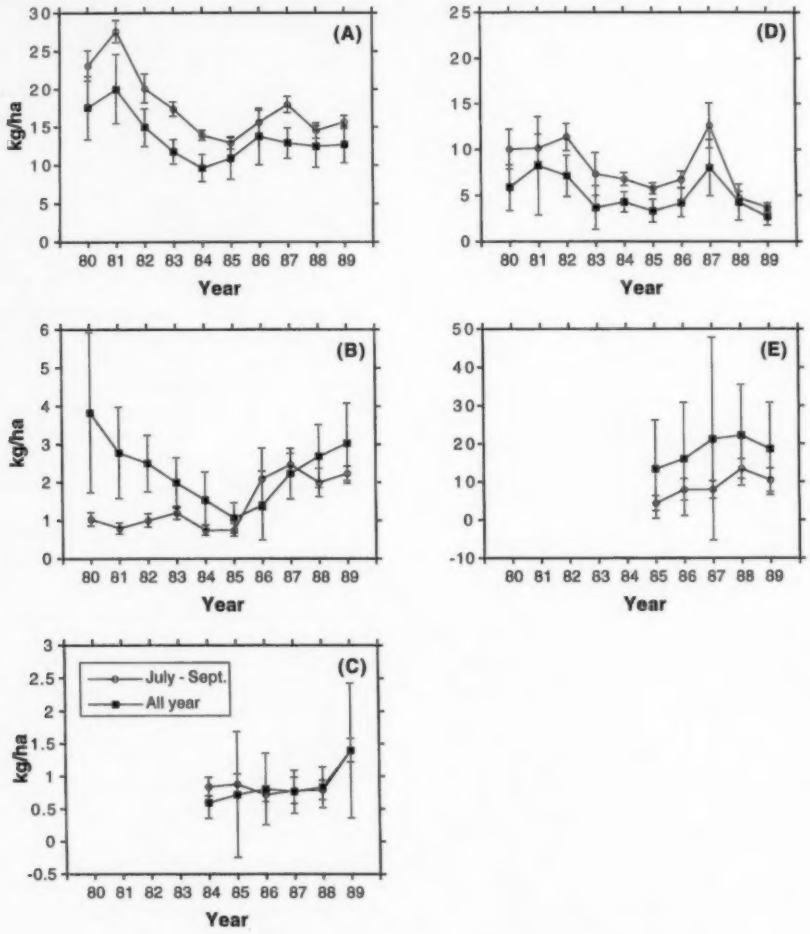

Table 8. Estimated discard rates used for the most recent stock assessments of the species analyzed in this study (data from Pikitch et al. 1988; Sampson 1993; Tagart 1993; Ianelli et al. 1994; Methot et al. 1994; Turnock et al. 1994).

	Years	Discard %
Dover sole	1982–1994	5
Sablefish	1982–1987	31
	1988–1992	25
English sole[a]	1977–1984	18
	1985–1992	12
Yellowtail rockfish	1985–1992	16
Shortspine thornyhead	1980–1987	13–17
	1988–1989	8

[a]Discard of female fish only.

could be scaled for comparison with research estimates. For the species we selected, discard was assumed to be consistent or to vary by a small amount during the 1980s (Table 8). This assumption may be invalid, however, for species with stringent catch quotas or by-catch regulations (Pikitch et al. 1988; Pikitch 1991). This problem can be minimized with the adoption of fishery observer programs.

A critical assumption in the use of any trawl surveys is the fishing efficiency of the gear and resulting catchability of fishes. For estimating biomass, NMFS assumed a catchability of 1.0 for their surveys, indicating that all fish in the path of a trawl would be caught and there is no herding effect (Methot et al. 1994). We made the same assumption for commercial trawls. For short periods, constant catchability may be a valid assumption. Over longer periods, such as a decade or more, the assumption of constant catchability is probably invalid. Kimura (1981), for example, reported that echo sounders and improved roller gear doubled the estimated efficiency of commercial vessels. Catchability should be studied periodically to ensure that CPUE estimates are comparable from year to year. A multiplicative model, such as the ones used by Gavaris (1980), Kimura (1981), or Large (1992), may then be

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needed to account for variability associated with differences in gear.

It is apparent that commercial fishery logbook data provide a comprehensive source of information because of the broad temporal and spatial fishing patterns of the fleet. For the period 1980–1989, estimates of fish distribution and relative abundance derived from logbook data closely resembled those from NMFS triennial trawl research data. Commercial fishery and research survey estimates of biomass were similar for species that are well sampled by trawl nets. These results suggest that information derived from logbooks can augment research data and improve estimates of the distribution and relative abundance of commercial fish species.

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Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability

Daniel E. Ruzzante, Christopher T. Taggart, and Doug Cook

Abstract: Polymorphism at six microsatellite DNA loci among cod larvae sampled repeatedly over a 3-week period from an aggregation on Western Bank of the Scotian Shelf provided evidence of several heterogeneous groups within the aggregation. There was strong evidence of heterozygote deficiency and departure from Hardy–Weinberg expectations for the larval aggregation as a whole ($N = 1337$) and for all larvae sampled within a single water mass (CW larvae), but not for a subset of these larvae considered to be part of a single cohort on the basis of age at length. These results suggest that both the entire aggregation and the CW subset originated from several distinct spawning events involving spawners with heterogeneous allelic compositions, but that the larvae forming the cohort originated from a single spawning event. Our results establish a link between the ecological match–mismatch hypothesis and the genetic "sweepstakes" selection hypothesis. There was no evidence that the larvae originated from different populations as measured by $(\delta\mu)^2$ distance, R_{ST}, and F_{ST} estimates among subsets. Additional analyses showed the larval cohort to have greater genetic similarity to adult cod sampled on Western Bank 2 years later than to adult cod sampled on Banquereau Bank (≈150 km away) also 2 years later. These results suggest that the genetic composition of cod on Western Bank remains stable over time.

Résumé : Le polymorphisme observé à six loci de microsatellites de l'ADN chez des larves de morue échantillonnées de façon répétée, pendant une période de 3 semaines, sur le banc Occidental de la plate-forme néo-écossaise a prouvé l'existence de plusieurs groupes hétérogènes dans cette concentration de larves. Les indications étaient fortes d'un défaut d'hétérozygotie et d'un écart par rapport à la loi de Hardy–Weinberg pour l'ensemble de la concentration de larves et pour toutes les larves échantillonnées dans une seule masse d'eau (eau de crête, larves CW), mais non pour un sous-ensemble de ces larves considéré comme faisant partie d'une seule cohorte d'après l'âge selon la longueur. Ces résultats permettent de penser que la concentration entière et le sous-ensemble de larves CW provenaient de plusieurs épisodes distincts de fraye impliquant des géniteurs présentant des compositions alléliques hétérogènes, mais que les larves formant la cohorte provenaient d'un seul épisode de fraye. Nos résultats ont établit un lien entre l'hypothèse écologique bon assortiment – erreur d'assortiment et l'hypothèse génétique de sélection « sweepstake. » Rien n'indiquait que les larves provenaient de populations différentes, d'après les estimations de la distance $(\delta\mu)^2$, de R_{ST} et de F_{ST} entre les sous-ensembles. Des analyses additionnelles ont montré que la cohorte de larves présentait une plus grande similarité génétique avec les morues adultes échantillonnées sur le banc Occidental 2 ans plus tard qu'avec les adultes capturés sur le Banquereau (≈150 km de distance) également 2 ans plus tard. Ces résultats permettent de penser que la composition génétique de la morue du banc Occidental reste stable au fil du temps. [Traduit par la Rédaction]

Introduction

The population genetic structure of a marine species is in part determined by recruitment variation in time and space. Information on the underlying causes of such variation can only be obtained by examining ecological and genetic changes occurring at the spatial and temporal scale of the oceanographic processes (mixing, advection, diffusion, etc.) directly influencing the distribution and abundance of the population under study; i.e., resolving biological processes at or below the scales of the physically driven processes (Roughgarden et al. 1988; Taggart and Frank 1990).

Most marine animals with pelagic eggs or larvae are highly fecund and abundant species that exhibit large variation in recruitment. They also show major discrepancies between observed and effective population size (Hedgecock 1994). The large recruitment fluctuations frequently observed among such species are generally attributed to factors affecting early life stages (Hjort 1914; Cushing 1972; Houde 1987; Peterman et al. 1988; Taggart and Frank 1990). When fertilization is external, its success will depend on oceanographic processes that vary spatially and temporally and thus affect gonad maturation rates, spawning time, and the dispersal of sperm and ova (Levitan and Petersen 1995). These same processes can equally affect the survival of postfertilization stages, for example, by

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directly transporting eggs and larvae to, or away from, nursery grounds or suitable settling areas (Iles and Sinclair 1982; Roughgarden et al. 1988; Possingham and Roughgarden 1990; Farrell et al. 1991).

Several hypotheses have been offered to explain the large discrepancies between observed and effective population numbers (reviewed in Hedgecock 1994). These include (*i*) large disparities between contemporary and historical (or long term) effective population sizes (i.e., bottleneck effects; Hedgecock et al. 1982; Nei and Graur 1984; Avise et al. 1988; Palumbi and Wilson 1990), (*ii*) diversity-reducing selection (Nei and Graur 1984), and (*iii*) large variance in the number of progeny among families (Hedgecock et al. 1982; Avise et al. 1988; Palumbi and Wilson 1990). In particular, Hedgecock (1994) suggested that the large disparity between effective and observed population sizes of very abundant marine animal species results from a large variance in reproductive output among individuals. In extreme cases the entire population may be replaced by the offspring of a small minority of individuals who, by chance ("sweepstakes"), happen to match their reproductive activity with ". . . oceanographic conditions conductive to spawning, fertilization, larval development and recruitment" (Hedgecock 1994, p. 124). Under this hypothesis, which is clearly linked to Cushing's (1972) match–mismatch hypothesis, many individuals fail to contribute to recruitment. This results in high population variance in offspring number and thus in much lower effective than actual population size.

High variance among individuals in their contribution to recruitment can result from varying degrees of synchrony in the release of sperm and ova (which translate into variance in fertilization success), from moderate, or even minor, differences in the timing of spawning among groups of mature individuals, and (or) from differences in access to essential resources by the offspring (i.e., match–mismatch; Cushing 1972). Thus, any biotic, oceanographic, or meteorological event (e.g., changes in wind direction or intensity, changes in the turbulent field) affecting gamete concentration or larval survival has the potential to affect the offspring (potential recruits) of some families but not of others (Levitan and Petersen 1995). At a slightly larger scale this is equally applicable to some cohorts, but not others. Minor timing differences in the production of different cohorts can result in large variance in their contribution to recruitment, and if there are differences in the allelic composition among spawning groups, then there should be detectable genetic differences among their offspring.

Of equal importance, and directly related to the issue of temporal and spatial genetic heterogeneity within a large planktonic larval aggregation, is the question of whether or not there is population structure, and if so, at what scale it is resolved. Although different techniques vary in their ability to detect population structure, no technique applied thus far to cod (i.e., allozymes, mitochondrial DNA, nuclear DNA restriction fragment length polymorphisms) has consistently, and in a complementary manner, shown evidence of population subdivision at other than large geographic scales, such as across ocean basins, or across deep oceanic channels (see Cross and Payne 1978; Mork et al. 1982, 1985; and Dahle and Jørstad 1993 for evidence using allozymes; Smith et al. 1989; Carr and Marshall 1991*a*, 1991*b*; Árnason and Rand 1992; Árnason et al. 1992; Pepin and Carr 1993; and Carr et al. 1995 for evidence using mitochondrial DNA; and Pogson et al. 1995 for evidence using nuclear DNA restriction fragment length polymorphism, RFLP).

In this study we describe the temporal changes at six microsatellite loci in a larval cod (*Gadus morhua*) aggregation tracked and sampled repeatedly on Western Bank of the Scotian Shelf over 3 weeks in November and December 1992. We demonstrate the existence of genetically heterogeneous groups (cohorts) of larvae within the aggregation. This suggests that the aggregation was composed of larvae that originated from different spawning events involving individuals with different allelic compositions. We then compare five microsatellite loci among larvae, which on the basis of size were presumed to form part of a single cohort, and adult cod collected locally in the same general area as the larvae and in a neighbouring location (≈150 km away) on the Scotian Shelf (Banquereau Bank). We show that it is possible to distinguish the cohort larvae as distinct from adult cod sampled on Banquereau Bank, but not as distinct from adult cod sampled locally on Western Bank, providing evidence that the genetic composition of cod on Western Bank remains stable over time.

Materials and methods

Field collections and laboratory procedures

Larval cod samples were collected during the *Petrel V* cruise 31 on Western Bank (Fig. 1*a*) during the period (Coordinated Universal Time (UTC)) 23 November to 16 December 1992 (Griffin and Lochmann 1993). Three sampling transects were conducted across the crest of Western Bank on 23–25 November to ascertain the distribution and abundance of cod larvae (Fig. 1*b*) and collect hydrographic data. This survey revealed several water masses in the region (Griffin and Thompson 1996; Taggart et al. 1996): (*i*) crest water (hereafter CW), on the crest of the bank, which was relatively well mixed and of intermediate temperature (9.1°C) and salinity (31.6 practical salinity units (psu)), (*ii*) a convergent thermohaline front on the eastern and southeastern margin of the CW (hereafter FRONT), and (*iii*) a well-stratified water mass having a surface lens of relatively cold fresh water surrounding the CW (hereafter OUT).

The CW was contained within a gyrelike feature (approximately 20 km in diameter) that rotated around the crest of Western Bank (Sanderson 1995)[2] and was tracked between 25 November and 16 December (Griffin and Thompson 1996). During this period the resident cod larvae within CW were sampled more or less continuously (Taggart et al. 1996). The water mass was tracked with the Ocean Probe real-time oceanographic monitoring system (see Bowen et al. 1995 for complete details), which included shipboard and moored telemetering instrumentation as well as telemetering Loran-C drifters. The Ocean Probe system delivered oceanographic data in real time to a data-assimilative hydrodynamic model (Griffin and Thompson 1996) that produced time-dependent flow fields within a 60 × 60 km model domain of the study area (Fig. 1). By employing this model (updated at least twice daily) in a Lagrangean frame of reference the flow fields could be used to assess the present, and predict the future, positions of previous sampling locations as they were advected around and across the bank within the moving water mass. Thus, we were able to track the aggregation of larval cod found within the CW.

Cod larvae were collected in the tracked CW and in the surrounding water masses every 4–8 h from 25 November (day 3) to 3 December (day 11, Fig. 1*c*) using either a 60-cm diameter bongo net sampler

[2] Sanderson (1995, p. 6762) erroneously reported his analysis as over the period 5–11 November 1992 when his analyses actually covered the period 25 November to 1 December 1992.

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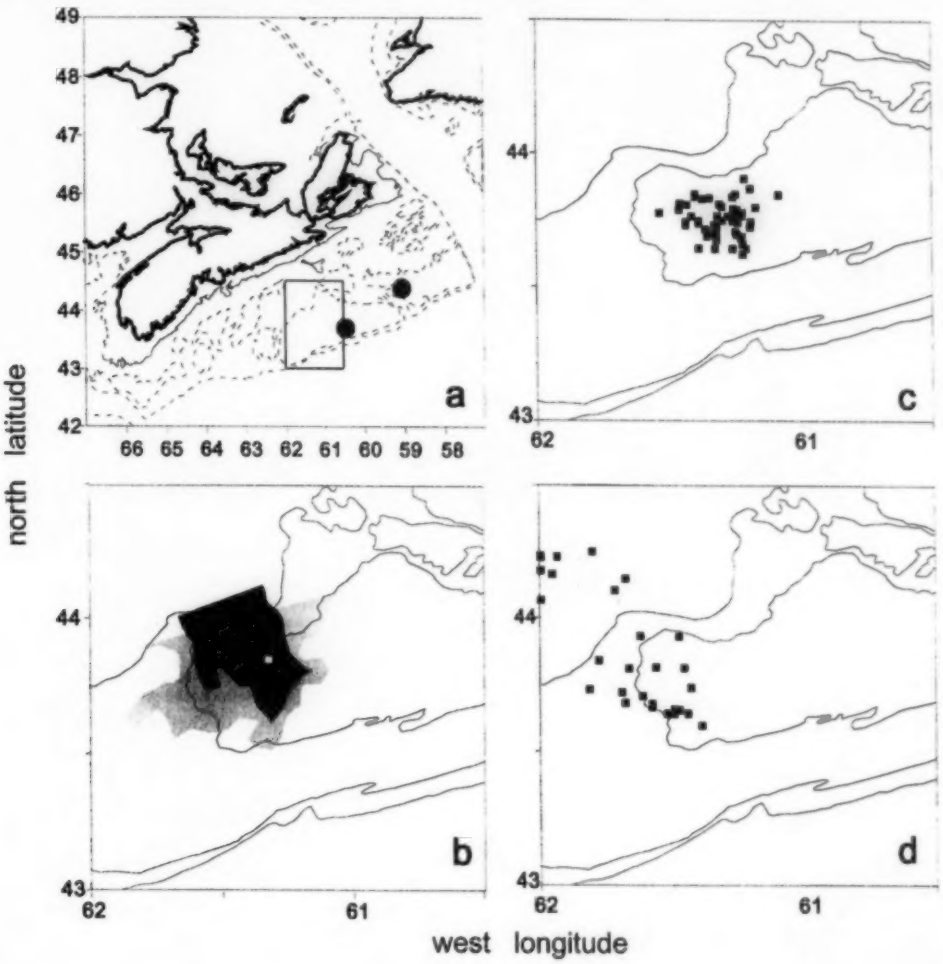
Fig. 1. Bathymetric charts of (*a*) the Scotian Shelf (100- and 200-m isobaths) showing the location of Western Bank (*b*, *c*, and *d* represent the area inside the box in *a*) and the median sampling locations (solid circles) of adult cod on Western and Banquereau banks in 1994. (*b*) Water-column larval cod concentration isopleths (graded grey-scale of 10, 20, and >30 cod larvae/100 m^3 on Western Bank (60-, 100-, and 200-m isobaths) as observed during 23–25 November 1992 and the location (central open square) of the centroid of the drifter cluster at the start of water mass tracking. (*c*) Larval cod sampling locations within the crest water mass during the prestorm period of tracking (25 November to 3 December 1992). (*d*) Larval cod sampling locations within the crest water mass during the poststorm period of tracking (10–16 December 1992).



fitted with 333-μm mesh nets (Posgay and Marak 1980) or a 1-m^2 Eastern Marine Marsh EZNET designed after the BIONESS (Sameoto et al. 1980) and fitted with ten 333-μm mesh nets. The bongo was towed at a nominal speed of 1 m/s in a continuous double oblique manner down to 5 or 10 m above the bottom for 10–15 min. The EZNET was also towed at a nominal speed of 1 m/s and was used to sample discrete depth strata at 5-m intervals, down to 35 m, and at 10-m intervals to within 10 m of the bottom. On recovery the nets were rinsed to the cod ends and plankton samples were immediately sorted for cod larvae. At least 10 larvae from each net were videotaped through a dissecting microscope (Miller et al. 1995) for morphometric analysis and individually preserved in liquid nitrogen for biochemical and genetic analyses.

A major storm caused a break in sampling between 4 and 7 December (days 12–15), although real-time monitoring and data assimilation were maintained to continue tracking of the CW (Griffin and Thompson 1996). Following the storm, a second survey of the bank crest area was conducted between 8 and 9 December (days 16–17) and was centred in the area where, according to the assimilated data and model output, a remnant of the CW sampled during the earlier portion of the cruise was to be found. Cod larvae were again collected within the remnant water mass, using the same sampling techniques described above, from 10 to 16 December (days 18–24) when a second storm dispersed the tracked water mass northward and off the bank, ending the study (Fig. 1*d*).

The larval concentration in the water column for each deployment was estimated by dividing the total number of cod larvae collected by all nets in a single deployment by the total volume of water filtered by all nets to produce a single estimate (Fig. 2). The standard lengths (SL, in millimetres) of larvae were measured by the protocol of Miller

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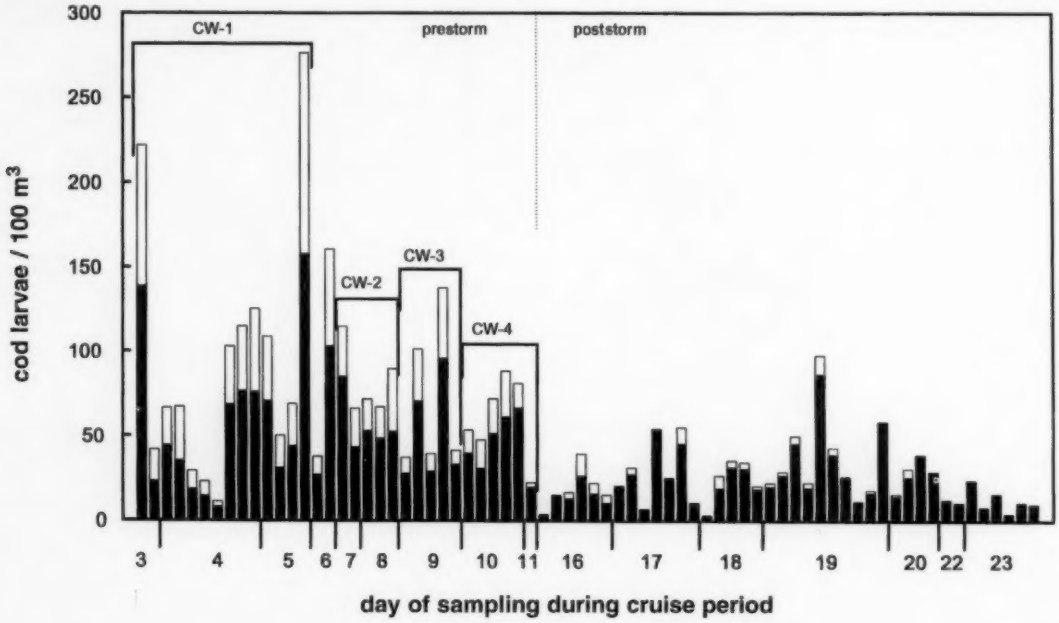
Fig. 2. Larval cod concentrations (larvae/100 m^3) observed within the crest water mass on Western Bank during days 3–23 (water mass tracking) of the pre- and post-storm cruise periods. Light shading represents the concentration of larvae that were considered part of the age at length cohort defined for the entire cruise period. CW-1 to CW-4 show concentrations for each of the four consecutive larval groups within the crest water mass (cohort and noncohort). The four consecutive groupings of the age at length cohort (cohort 1 to cohort 4) are subsets of the same four CW groups. Note that the day of sampling interval is proportional to the number of samples taken in a day in the crest water and not to the elapsed period.



et al. (1995). An age (in days post-hatch; *d*) was assigned to each larva according to an age at length relationship established for cod larvae collected in December on the Scotian Shelf (M. Meekan, Department of Biology, Laval University, Quebec, QC G1K 7P4, personal communication) and was used to select individuals from a common cohort within the tracked CW parcel:

$$(1) \quad d = 1222(6.47 \times 10^{-9})^{\frac{1}{SL}} SL^{-0.63}$$

We also sampled subadult and adult cod in July 1994 from the western end of Banquereau Bank ($N = 48$) in the vicinity of The Gully (median sample location at 44.4°N, 59.09°W; Fig. 1*a*) and in the vicinity of Western Bank ($N = 48$; median sample location 43.57°N, 60.47°W; Fig. 1*a*) during the annual research vessel survey conducted by the Canada Department of Fisheries and Oceans (Anonymous 1994). These cod ranged in size between 23 and 77 cm in fork length (median 37 cm for Western and 39 cm for Banquereau) and were between 2 and 8 years old (median age: 4 years in both locations). The majority of spawners (ages 4+) were recently spent or recovering from spawning at the time of collection.

Tissue collections and processing

Cod larvae initially preserved in liquid nitrogen were thawed and the eyeballs removed for genetic analysis (the remainder of the carcass was used for other phenological and biochemical purposes). The eyeballs were placed in 50 µL of extraction buffer (10 mM Tris, pH 8.3, containing 50 mM KCl and 0.8% Tween 20) and held at –20°C. The DNA was released from the tissue by digestion with proteinase K (200 µg/mL final concentration) at 65°C overnight. Following digestion the samples were incubated at 95°C for 15 min to deactivate the proteinase K and 1–2 µL of this mixture was used directly for polymerase chain reaction (PCR).

The DNA from adult cod (measured for length, weighed, and later aged) was obtained from either blood or soft muscle tissue taken from the tongue. Samples were preserved immediately in 5 mL of 95% ethanol and stored.

Details of DNA extraction from alcohol-preserved tissue are in Bentzen et al. (1990). Briefly, a sample of approximately 100 mg of muscle or 100 µL of blood in ethanol was washed in high TE (100 mM Tris-HCl, 40 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) to remove the ethanol. The samples were then placed in 250 µL of lysis buffer (10 mM Tris-HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulphate (SDS) and 400 µg/mL proteinase K). Samples were digested at 65°C for 18 h and transferred to an SST tube (Becton Dickinson). The samples were extracted twice with 2.5 mL of phenol buffered with 0.1 M Tris, pH 8.0, and once with 2.5 mL chloroform. The supernatant containing the purified nucleic acids was transferred to a 1.5-mL Eppendorf tube and precipitated with 0.2 M NaCl and 1 volume of isopropanol. The DNA pellet was resuspended in 100 µL of TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) and adjusted to 25 ng/µL as a PCR template.

Genetic analysis

PCR analysis was as previously described by Brooker et al. (1994). We employed six sets of cod microsatellite primers: Gmo2, Gmo4, Gmo120, Gmo132, Gmo141, and Gmo145 (Table 1). Probes Gmo2, Gmo4, Gmo120, Gmo132, and Gmo141 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The allele sizes of all loci varied by increments of two base pairs (bp) except for Gmo145. Gmo145 showed occasional alleles that differed by a single bp change. The PCR products were resolved on 6.5%

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Table 1. Sequence variants in the *F′* and *R′* ends that define the six different cod microsatellite loci along with their respective annealing temperatures and the primary references for their application.

Locus	Sequence (5′–3′)	Annealing temperature (°C)	Source
Gmo2	$(GT)_x$	51	Brooker et al. 1994
F′	CCC TCA GAT TCA AAT GAA GGA		
R′	GTG TGA GAT GAC TGT GTC G		
Gmo4	$(GT)_x$	57	Wright 1993
F′	ATG TTT AGG CAG ATT TGA ATC AGC		
R′	TGT GGG TAT GTG CAA AGC ATA CAT		
Gmo120	$(GT)_x$	54	Ruzzante et al. 1996
F′	GAG CAA ACA TGC TCA GAG TG		
R′	GAC TGA TCT CCA TGA GAG G		
Gmo132	$(GT)_x$	52	Brooker et al. 1994
F′	GGA ACC CAT TGG ATT CAG GC		
R′	CGA AAG GAC GAG CCA ATA AC		
Gmo141	$(GT)_x$	51	This study
F′	CCG AAT ACT GTT TGA TTG GC		
R′	GAC ATA CTA CTA GGA ACA TAG		
Gmo145	$(G)_x(GA)_x$	50	Brooker et al. 1994
F′	GCA TTG TAG GAA CAA TTA AC		
R′	GTG CAT GTG CTC ATT ATA GC		

Table 2. Levels of genetic variation in an aggregation of larval cod (*Gadus morhua*) on the Scotian Shelf at six microsatellite loci.

Group	Mean sample size per locus (individuals)	Total alleles	Mean H_{obs}	Mean H_{exp}	Deficiency	
					Mean *D*	*p*
Pool	1226.8	276	0.895	0.902	–0.009	0.015
Pool before	968.3	267	0.893	0.902	–0.011	0.005
CW	939.8	268	0.894	0.902	–0.009	0.018
CW before	705.7	256	0.891	0.901	–0.013	0.008[a]
CW-1 (days 3, 4, 5)	132.0		0.909	0.900	0.011	0.273
CW-2 (days 7, 8)	197.2		0.888	0.905	–0.020	0.023
CW-3 (day 9)	203.0		0.892	0.896	–0.005	0.210
CW-4 (days 10, 11)	213.0		0.889	0.898	–0.011	0.083
Cohort	304.2	216	0.898	0.899	–0.003	0.279
Cohort before	283.3	212	0.897	0.898	–0.004	0.263

Note: Pool, the pool of larvae collected in the tracked crest water (CW), in the thermohaline front (FRONT), and in the surrounding water mass (OUT); pool before, the pool of larvae (CW + FRONT + OUT) collected before the storm that interrupted sampling; CW-1 to CW-4, the four consecutive subsets of CW larvae collected before the storm; cohort, the subset of CW larvae defined to be part of a cohort on the basis of age at length and sampling date information; cohort before, cohort larvae collected before the storm; mean D, deficiency (if <0) of heterozygotes averaged over six loci. Deficiencies of heterozygotes for individual loci are presented for the CW before group (see footnote *a*).

[a]Deficiency values for individual loci were as follows: Gmo2, –0.035; Gmo4, 0.016; Gmo120, 0.010; Gmo132, –0.032; Gmo141, –0.036; Gmo145, –0.001.

sequencing gels and the alleles were sized relative to a sequence ladder generated from M13mp18 (Yanisch-Perron et al. 1985).

Biological data

Cod larvae were classified according to sampling date and the water mass from which they were collected (Taggart et al. 1996), i.e., (*i*) inside the well-mixed crest water (CW), (*ii*) at the front (FRONT), or (*iii*) in the surrounding stratified water mass (OUT).

We examined the genetic variation at the six microsatellite DNA loci in a maximum of 1337 cod larvae (Table 2), and at five of these loci in the 96 adult cod samples. The genetic analysis of the larval aggregation proceeded at several scales of temporal and spatial resolution. We first analyzed the pooled data set (i.e., all larvae from CW, FRONT, and OUT collected over the whole time period) as well as the fraction of the pooled data (CW + FRONT + OUT) collected only prior to the storm that interrupted sampling. Subsequent analyses focused only on samples from the CW (the tracked water mass) where we subdivided the CW larvae that were collected before the storm (Fig. 2) into four temporally sequenced groups of similar sizes (CW-1 to CW-4; Table 2). These four groups generally corresponded to different larval concentration maxima observed in the crest water over the prestorm period (Fig. 2).

To assess the temporal changes in the genetic profile of a single cohort within the tracked CW we considered a subgroup of these larvae, i.e., those that hatched during a specific period. On the basis of the age at length relationship described in (*i*), larvae that were at least 1 day old (post-hatch) and no more than 6 days old on day 3 were considered part of the initial cohort. This hatch window was then stepped through time, such that a larva collected 3 days later (day 6) was considered part of the same cohort if its length indicated it to be between 4 and 9 days old. Larvae from this cohort collected before the storm were further subdivided into four consecutive groups (cohort 1 to cohort 4, Fig. 2) that were drawn from the four subsets of CW larvae (Fig. 2).

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Table 3. Single locus statistics.

Locus	N (individuals)	n (alleles)	Size range (bp)	H_{obs}	H_{exp}	Probability of homogeneity of allele frequency distribution: Pool before – pool after	CW before – CW after	CW, 4 groups	Cohort, 4 groups
Gmo2	1303	29	102–204	0.774	0.794	0.042	0.061	0.029	0.051
Gmo4	1000	61	111–269	0.970	0.960	0.003*	0.006*	0.449	0.234
Gmo120	1227	44	108–230	0.958	0.952	0.111	0.084	0.290	0.159
Gmo132	1315	31	101–187	0.777	0.798	0.003*	0.008*	<0.001**	0.006*
Gmo141	1285	63	103–243	0.945	0.970	0.180	0.266	0.006*	0.027
Gmo145	1231	48	137–233	0.943	0.942	0.113	0.112	<0.001**	0.019

Note: N, number of individuals; n, number of alleles; size range, allele size range in base pairs (bp); H_{obs}, observed heterozygosity; H_{exp}, expected heterozygosity. The last four columns provide probabilities of homogeneity of allele frequency distributions between or among the groups listed. As expected for microsatellite DNA, heterozygosity estimates were high and ranged from 0.794 for a locus with a low number of alleles (e.g., Gmo2) to 0.970 for a locus with a high number of alleles (e.g., Gmo4).

*$p < 0.008$ with Bonferroni adjustment for six simultaneous comparisons.

**$p < 0.002$ with Bonferroni adjustment for six simultaneous comparisons.

Data analysis

We conducted tests for heterozygote deficiency or excess as well as for the probability of homogeneity of allele frequency distribution between and among the samples collected on different dates during the sampling period. Tests for Hardy–Weinberg equilibrium (HWE) were conducted using the goodness of fit and log-likelihood ratio tests following Weir (1990, pp. 84–85). We estimated $(\delta\mu)^2$ (Goldstein et al. 1995). Estimates of subpopulation structure were obtained using R_{ST} (Slatkin 1995), and for comparative purposes we calculated F_{ST} values following Weir and Cockerham (1984).

Significance values for all tests (i.e., heterozygote deficiency, HWE, homogeneity of allele frequency distributions, genetic distances, and estimates of subpopulation structure) were determined by Monte Carlo simulations and the bootstrap method (at least 1000 resampling trials per individual comparison, Manly 1991). In all cases, bootstrapping was conducted by reshuffling alleles across individuals and populations for each locus separately. Bootstrapping each locus independently allows comparison of estimates across loci. Bootstrapping across individuals and populations allows estimation of significance values for the test under consideration (e.g., HWE, R_{ST}). This method addresses the variance associated with statistical sampling but not that associated with genetic sampling, which occurs each new generation during reproduction (Weir 1990). The standard method of accounting for the effects of genetic sampling is to bootstrap across loci, but this method is meaningful only if a large number of loci is available. We have assumed that the six microsatellite DNA loci examined here constitute a random sample of the genome. Estimates of R_{ST} and F_{ST} combined over loci were calculated by first averaging numerators and denominators across loci and then taking ratios, as suggested by Weir and Cockerham (1984) and Slatkin (1995).

Results

The average sample size per locus for the pooled data was 1226.8 larvae (Table 2) with a minimum of 1000 for Gmo4 and a maximum of 1315 for Gmo132 (Table 3). The majority of these larvae were sampled in the crest water (CW) mass (mean sample size per locus: 939.8 larvae; Table 2) and most of these were sampled before the storm (days 3 through 11 of the cruise period, mean sample size per locus: 705.7, Table 2). There were a total of 276 alleles in the pooled data and the majority were present in the subset of larvae collected in the CW prior to the storm ($n = 256$ alleles, Table 2). As expected for microsatellites, observed and expected heterozygosities were high and ranged from 0.878 to 0.909 and from 0.896 to 0.905, respectively (Table 2).

Temporal changes in microsatellite DNA variation

There were significant deficiencies of heterozygotes in the larval collection as a whole (CW, FRONT, and OUT) and in the subset collected in the crest water mass (CW) when the entire sampling period was considered, as well as when only the period prior to the storm was considered (Table 2). In contrast, there was no evidence of heterozygote deficiency among the subset of CW larvae that were considered to be part of a single cohort (Table 2).

Similar analyses within each of the four temporally sequenced groups of CW larvae (regardless of cohort affinity) also showed evidence of heterozygote deficiency for three of the four groups (although the results are significant for only one group ($p = 0.023$) and marginal for a second group (Table 2). In contrast, we found no evidence of heterozygote deficiency in any of the four temporal groups that constituted the age at length cohort (cohort 1 to cohort 4, $p > 0.123$).

Tests of HWE using the goodness of fit and the log-likelihood ratio tests were consistent with the above results. The analyses indicated departure from equilibrium ($\alpha' = 0.008$ with Bonferroni adjustment for six loci) with both tests for the six loci for all larvae (pool) as well as for all larvae collected before the storm (pool before). Larvae collected in the CW prior to the storm showed evidence of departure from HWE with both tests for five loci, and with one test for the remaining locus (Gmo4). In contrast, both tests showed that for all but one locus (Gmo2) the larval cohort as a whole was in HWE.

Microsatellite DNA variation among loci

Analyses based on genetic variability at individual loci did not allow rejection of the null hypothesis that the larval cohort originated from a single group of spawners. However, the same analysis with all of the CW larvae (regardless of cohort affinity) resulted in rejection.

Allele frequency distributions were heterogeneous for two of the six loci (Gmo4 and Gmo132) when all larvae collected before the storm were compared with all larvae collected after the storm (Table 3: pool before – pool after). The same was true for the CW subset (Table 3: CW before – CW after), suggesting

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Table 4. Probability of homogeneity of allele frequency distributions among three groups: the entire larval cohort or each of the four consecutive subsets collected before the storm (cohort 1 to cohort 4), and adult cod collected on Banquereau Bank ($N = 48$), and on Western Bank ($N = 48$).

Locus	Entire cohort ($N = 316$)	Cohort 1 ($N = 79$)	Cohort 2 ($N = 86$)	Cohort 3 ($N = 74$)	Cohort 4 ($N = 77$)
Gmo2	0.017	0.007*	0.005*	0.014	0.038
Gmo4	0.692	0.831	0.264	0.315	0.782
Gmo120	0.576	0.434	0.151	0.578	0.269
Gmo132	0.003*	<0.001**	0.001**	<0.001**	0.004*
Gmo145	0.115	0.052	0.061	0.165	0.007*

Note: The values in the table are for comparisons among three groups (cohort, Banquereau Bank adults, Western Bank adults).

*$p < 0.010$ with Bonferroni adjustment for five simultaneous tests.

**$p < 0.002$ with Bonferroni adjustment for six simultaneous comparisons.

that the composition of the larval aggregation, even that of the subset collected within a single water mass, changed during the storm period. Differences were also significant among the four consecutive CW larval subsets (CW-1 to CW-4) collected before the storm, i.e., these subsets were different at three loci (Gmo132, Gmo141, Gmo145; Table 3: CW, 4 groups). By contrast, the four consecutive cohort subsets (cohort 1 to cohort 4) were different at only one locus (Gmo132; Table 3: cohort, 4 groups).

Genetic distances and population structure in the larval aggregation

Although the above results based on allele and genotype frequency distributions suggest that the entire larval aggregation in the CW originated from different spawning groups, results based on $(\delta\mu)^2$ distances and on estimates of population structure (R_{ST}) suggest that the different spawning groups may not have come from different populations but instead may have been different components of a single population.

None of the six pairwise comparisons among the subsets of the CW larvae or of the cohort larvae indicated significant differences ($\alpha' = 0.008$ after Bonferroni adjustment for six simultaneous groups) when measured with $(\delta\mu)^2$ distance ($p \geq 0.106$ for comparisons between the CW subsets, and $p \geq 0.047$ for comparisons between the cohort subsets). Similarly, there was no evidence of significant population structure between or among any of the subsets of larvae when measured with R_{ST} ($p \geq 0.245$) or with F_{ST}.

Genetic distances and population structure: the larval cohort and adult cod

We compared the genetic composition of the larval cohort with that of two subadult and adult cod samples collected on the Scotian Shelf in July 1994 (one sample from Western Bank and one from the eastern end of Banquereau Bank). Genetic distances and estimates of population structure (R_{ST} and F_{ST}) consistently indicated that the larval cohort was more similar to the cod collected in the same general location on Western Bank than to those collected on Banquereau Bank.

We compared variation in five of the six microsatellite loci (Gmo141 was not run for the adult cod samples). The allele frequency distributions for Gmo132 were consistently heterogeneous among all three populations, either for the entire cohort sample, or when any of its four consecutive subsets were considered (Table 4). Probabilities that allele frequencies were homogeneous were low and occasionally significant for two other loci: Gmo2 and Gmo145. Similar analyses comparing the cohort larvae separately, with the Banquereau or the Western Bank adult cod samples, indicated that the cohort larvae differed more from Banquereau Bank cod than from Western Bank cod. Only 3 out of 20 possible comparisons (five loci, four subsets) among the larvae and Western Bank cod indicated heterogeneity in allele frequency distribution ($\alpha' = 0.01$ with Bonferroni adjustment for five simultaneous tests, i.e., five loci), whereas 6 out of 20 comparisons indicated heterogeneity between larvae and Banquereau Bank cod (data not shown). This difference is not great. However, $(\delta\mu)^2$ genetic distance estimates also suggest that the larval cohort was more similar to cod from Western Bank than to those from Banquereau Bank (Table 5), and, as well, comparison of R_{ST} and F_{ST} estimates of population structure between the cohort larvae and each of the adult populations (Table 6) also showed that the larvae were consistently more similar to Western Bank cod than to Banquereau Bank cod. Thus, all four measures consistently indicated greater affinity of the larvae to those adults collected on Western Bank.

Table 5. $(\delta\mu)^2$ distances between the entire larval cohort, each of the four consecutive subsets collected before the storm (cohort 1 to cohort 4), and adult cod collected on Banquereau and Western banks.

	Banquereau Bank ($N = 48$)		Western Bank ($N = 48$)	
Group	$(\delta\mu)^2$	p	$(\delta\mu)^2$	p
Entire cohort ($N = 316$)	5.89	0.041	2.25	0.359
Cohort 1 ($N = 79$)	6.22	0.030	4.98	0.070
Cohort 2 ($N = 86$)	5.82	0.033	2.11	0.416
Cohort 3 ($N = 74$)	4.43	0.093	1.31	0.672
Cohort 4 ($N = 77$)	11.81	0.006	6.12	0.097

Note: $p < 0.05$ for comparisons involving the entire cohort and $p \leq 0.012$ for comparisons between each of the four consecutive cohort subsets and adult cod with Bonferroni adjustment for four simultaneous tests. $(\delta\mu)^2$ distance between Banquereau and Western banks = 2.44 ($p \approx 0.450$).

Discussion

We have shown, on the basis of microsatellite DNA variation, that an aggregation of larval cod repeatedly sampled on the Scotian Shelf over approximately 3 weeks was formed by more than one genetically distinct larval group. Furthermore, a subset of the larval aggregation, presumed to be part of a single cohort on the basis of age at length, was genetically more similar to adult cod sampled some 2 years later in the same general area on Western Bank than to adult cod sampled on a neighbouring bank situated approximately 150 km away. These results therefore suggest that (*i*) the aggregation was formed by larvae that originated from different spawning events involving different groups of individuals and (*ii*) the genetic composition of cod on Western Bank remained fairly stable over time. We next address these points and their implications in detail.

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Table 6. R_{ST} and F_{ST} estimates of population structure between each of the four consecutive cohort subsets and adult cod collected on Banquereau Bank and on Western Bank.

	Cohort 1		Cohort 2		Cohort 3		Cohort 4	
Group	Estimate	p	Estimate	p	Estimate	p	Estimate	p
				R_{ST}				
Banquereau Bank	0.0322	0.005	0.0318	0.033	0.0149	0.110	0.0599	0.001
Western Bank	0.0180	0.043	0.0160	0.140	0.0012	0.468	0.0419	0.004
				F_{ST}				
Banquereau Bank	0.0010	0.241	0.0067	0.002	0.0027	0.107	0.0029	0.064
Western Bank	0.0011	0.276	0.0022	0.100	⩽0.00001	0.438	0.0019	0.142

Note: Sample sizes are as in Table 5.

Heterogeneity within the larval aggregation

The presence of several heterogeneous groups among the larvae collected in the tracked gyrelike water mass on the crest of the bank (CW) was suggested by evidence of heterozygote deficiencies (Wahlhund effect), departures from HWE, and heterogeneity of allele frequency distributions when all larvae sampled in the crest water (CW) mass were considered, but not when similar analyses were restricted to larvae that, on the basis of their estimated age at length, comprised a single cohort (Tables 2 and 3).

HWE is characteristic of large, randomly mating populations. A population is in HWE for a given locus if the probability of observing a given genotype is equal to the product of the probabilities of observing each of the alleles (i.e., the allele frequencies) for a homozygote, or twice this product for a heterozygous genotype. If a population is subdivided, and there are two or more groups that differ in allele frequencies at a given locus, then analysis of the entire population, without considering its structure, will show a deficiency of heterozygotes. This is essentially what our analyses described for the entire larval aggregation and the subset of all larvae sampled in the crest water mass: they appear to have originated from several spawning groups among which there were marked differences in allele frequencies. In contrast, there was no evidence for heterozygote deficiency (and evidence for departures from HWE was weak) among the cod larvae that formed a single cohort within the water mass. Comparisons of allele frequency distributions among possible consecutive subsets of larvae supported these conclusions; there were more significant differences in allele frequencies among the four consecutive subsets of all larvae sampled in the water mass than among the four consecutive subsets of the cohort (Table 3).

Departures from HWE can result from factors other than population subdivision, such as selection, inbreeding, phenotypic assortative mating, and (or) the presence of null alleles (Devlin et al. 1990; Chakraborty and Jin 1992). Population subdivision, however, is believed to be the most important of these factors for microsatellite (variable number of tandem repeats, VNTR) loci (Lander 1989). Null alleles are unlikely to be the cause of the deficiency of heterozygotes in the pooled data set or the subset of all larvae sampled in the crest water mass (Table 2). If null alleles were responsible for this deficiency it is reasonable to assume that they would be present among the larval cohort as well. The fact that we do not detect a deficiency of heterozygotes within the cohort suggests that, if present at all, null alleles are unlikely to be the sole cause of the departures from HWE or of the heterozygote deficiencies observed at the larger time and space scales of the larger groupings.

Although our results suggest the presence of several genetically heterogeneous groups of larvae within the larval aggregation collected over the sampling period, it is not trivial to estimate how many individuals might have contributed gametes to the formation of each of these groups. In a related study that attempted to infer possible kinship relationships among the same individuals as the ones used here, Herbinger et al. (1997) found no evidence of any temporal or spatial family structure within a subset of 315 individuals analyzed in detail. Assuming that these individuals were indeed representative of the 1400 or so larvae collected and assayed, they estimated the minimum parental population to have been of the order of 2800 individuals (1400 of each sex), since no two larvae could be assured of having two parents in common (Herbinger et al. 1997). We believe that actual population abundance was much higher, perhaps an order or two of magnitude higher. Calculations based on an exponential mortality model for the tracked cohort (Taggart et al. 1996) and the volume of the crest water mass suggest that the number of spawners necessary to produce the observed numbers of larvae may have been no less than 100 000. This conservative estimate assumed an average fecundity of 200 000 eggs per female (fecundity for a 50-cm cod; Scott and Scott 1988; somewhat larger than the cod sampled in the region in 1994, see methods), no egg mortality, complete fertilization, and a 1:1 sex ratio. The true spawning population size was likely higher than this estimate.

Population structure: the larval cohort and adult cod

Collectively, the results based on $(\delta\mu)^2$ distance and on estimates of population structure, R_{ST} and F_{ST} (Tables 5 and 6), suggest a fairly stable genetic composition of cod over time on Western Bank; i.e., the cohort in the larval aggregation was more similar to adult cod from Western Bank collected some 2 years later than to adult cod from the neighbouring Banquereau Bank. The existence of significant genetic differences between the cohort larvae and adult cod sampled only 150 km away in the area of Banquereau Bank is intriguing but perhaps not unique and could be explained as resulting from isolation by distance. The broader implications of these results are that, given moderate sample sizes (no less, and preferably more, than 50 individuals per location, D.E. Ruzzante, unpublished observations), if genetic differences exist in Atlantic cod at scales of hundreds of kilometres (e.g., among neighbouring banks), then they are likely to be detected with microsatellite DNA variation. Two related studies are consistent with these conclusions: in the first of these studies, Ruzzante (1996)

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used microsatellite DNA to distinguish cod overwintering inshore in a Newfoundland bay from those overwintering a few hundred kilometres away on the Grand Bank. Differences occurred despite the fact that offshore and inshore overwintering cod intermingle inshore during the summer feeding period. In the second of these studies, Bentzen et al. (1996) described evidence based on microsatellite DNA polymorphism suggesting that genetic differences exist among cod populations in the northwest Atlantic (i.e., among northern cod, Flemish Cap, and Scotian Shelf samples), and between northwestern and southeastern components of the northern cod complex off Newfoundland. Microsatellites, therefore, would seem to allow the detection of population structure at smaller spatial and temporal scales than has been possible to examine in the past with a variety of other genetic markers.

Previous studies on the genetic structure of Atlantic cod populations based on allozymes, mitochondrial DNA, and nuclear DNA RFLP appear not to have the same small-scale resolution afforded by microsatellite DNA polymorphism. One can argue that studies based on allozyme loci have produced ambiguous results because they tend to show significant differences among neighbouring populations when a limited number of blood protein loci are examined (Møller 1968; Jamieson and Otterlind 1971; Jamieson 1975; Cross and Payne 1978; Dahle and Jørstad 1993), but these differences sometimes disappear when a larger number of conventional electrophoretic loci are examined over the distributional range of the species (Mork et al. 1982, 1985). Studies of mitochondrial DNA variation have shown evidence of differentiation across the North Atlantic (Smith et al. 1989; Carr and Marshall 1991*a*; Árnason and Rand 1992), but not at smaller geographic scales (Carr and Marshall 1991*a*, 1991*b*; Árnason et al. 1992; Pepin and Carr 1993; but see Dahle 1991). In a recent study based on RFLP, Pogson et al. (1995) showed that these genetic markers are capable of detecting genetic population structure at ocean basin scales where allozyme loci do not.

Cohort contribution

We have shown that the larval aggregation on Western Bank was composed of genetically heterogeneous groups of larvae that originated from different spawning events. However, a subset of this aggregation, presumed to be part of a single cohort on the basis of age at length, remained genetically homogeneous through time.

Distinct larval cohorts within a large planktonic aggregation are found in other species. For example, Lambert (1984) reported evidence based on polymodal size distributions of a succession of distinct larval cohorts separated by periods of several days in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Lambert (1984) discussed the possibility that such reproductive strategy might be widespread among marine species with pelagic eggs and suggested it may have important consequences for the survival of early life stages by ". . . spreading the spawning effort over time to take advantage of a variable environment" (Lambert 1984, p. 1562).

The large larval aggregation described in the present study was confined within a relatively small area of coastal ocean involving three different water masses that contained larvae spanning a range of sizes and ages. Considered as a whole, the aggregation was genetically heterogeneous. However, a subset of these larvae, considered to be part of a cohort on the basis of age at size, was genetically homogeneous. Together, this evidence indicates that the larval aggregation as a whole resulted from the contribution of more than one and presumably several spawning groups who's offspring form genetically defined larval cohorts upon which temporally and (or) spatially varying biotic, oceanographic, and meteorological processes can act to effect differential reproductive success among individual spawners and (or) spawning groups. If our interpretation is correct, then moderate, or minor, differences in the timing of spawning among groups of mature individuals or differences in the availability of resources to the offspring (i.e., match–mismatch; Cushing 1972, see also Levitan and Petersen 1995) appear to be the mechanisms that lead to high variance among cohorts in their contribution to recruitment, and thus explain part of the relationship between observed and effective population sizes, as hypothesized by Hedgecock (1994).

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Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic

Paul Bentzen, Christopher T. Taggart, Daniel E. Ruzzante, and Doug Cook

Abstract: Allelic variation in six highly polymorphic microsatellite loci (mean heterozygosity, 86%) provided evidence that cod (*Gadus morhua*) in the northwest Atlantic belong to multiple genetically distinguishable populations, and further that genetic differences may also exist between northwestern and southeastern cod aggregations within the northern cod stock complex off Newfoundland. Cod were sampled from winter aggregations ranging from Hamilton Bank to the northern Grand Bank in the northern cod complex, and from the Flemish Cap, the Scotian Shelf, and the Barents Sea. Tests of allele frequency homogeneity (χ^2), F_{ST}, and $(\delta\mu)^2$, allele sharing, and Rogers' distance measures revealed significant differences among northern cod, Flemish Cap, Scotian Shelf, and Barents Sea samples. Within the northern cod complex, two pooled samples, NORTH (Hamilton, Funk, and Belle Isle banks) and SOUTH (northern Grand Bank area), were distinguishable using χ^2, $(\delta\mu)^2$, and allele sharing measures. Both $(\delta\mu)^2$ and Rogers' distances clustered western Atlantic samples in two groups distinct from the divergent Barents Sea sample; one comprised NORTH, SOUTH, and Scotian Shelf, and the other, Flemish Cap.

Résumé : La variation allélique de six loci de microsatellites fortement polymorphes (hétérozygotie moyenne de 86%) a mis en évidence le fait que les morues (*Gadus morhua*) du nord-ouest de l'Atlantique appartiennent à de multiples populations génétiquement distinctes, et qu'il peut en outre exister des différences génétiques entre les concentrations de morues du nord-ouest et du sud-est dans le complexe de stocks de morue du nord situé près de Terre-Neuve. Des morues ont été échantillonnées dans le complexe de stocks de morue du nord, du banc Hamilton au nord du Grand Banc; sur le Bonnet flamand; sur la plate-forme néo-écossaise; enfin, en mer de Barents. Les tests de l'homogénéité de la fréquence allélique (χ^2), de F_{ST} et des distances $(\delta\mu)^2$, du partage des allèles et de Rogers ont révélé des différences importantes entre les échantillons du complexe de morue du nord, du Bonnet flamand, de la plate-forme néo-écossaise et de la mer de Barents. Dans le complexe de morue du nord, deux échantillons combinés, NORD (bancs Hamilton, Funk et de Belle-Isle) et SUD (région du nord du Grand Banc) se distinguaient grâce au calcul de χ^2, de $(\delta\mu)^2$ et du partage des allèles. Le calcul des distances $(\delta\mu)^2$ et de Rogers regroupaient les échantillons de l'ouest de l'Atlantique en deux groupes distincts de l'échantillon particulier de la mer de Barents; l'un rassemblait les échantillons NORD, SUD et néo-écossais, l'autre le Bonnet flamand.
[Traduit par la Rédaction]

Introduction

Despite considerable study in the past, basic features of the population structure of Atlantic cod (*Gadus morhua*) remain controversial. Although spawning areas for cod in many parts of its North Atlantic range are known to be relatively discrete, stable over time, and often relatively distant from other spawning areas (Brander 1994; Serchuck et al. 1994; Frank et al. 1994; Taggart et al. 1994; Buch et al. 1994; Schopka 1994), whether or not such spawning aggregations constitute reproductively and genetically distinct populations remains uncertain. Proper resolution of these uncertainties is important for a detailed understanding of the biology of the species and ultimately for successful management (Angel et al. 1994). These issues are exemplified by the seriously depleted northern cod stock complex (Northwest Atlantic Fisheries Organization (NAFO) divisions 2J, 3K, and 3L).

The distribution and migration patterns of northern cod are complex. Most mature northern cod overwinter in warm (>2°C) slope water along the margin of the continental shelf, but some remain inshore all winter, relying on serum antifreeze proteins to survive in cold (<0°C) coastal water (Goddard et al. 1994). Spawning occurs over a 3- to 4-month period from winter through early summer, and generally begins earlier in the north than in the south (Myers et al. 1993). Spawning takes place in offshore bank areas and along the continental shelf break, but some also occurs in the interior of the Grand Bank and in coastal regions (reviewed in Taggart et al. 1994). Cod that have overwintered and spawned offshore migrate inshore in the spring to coastal feeding grounds, then return offshore in late autumn – early winter (Templeman 1966). Juvenile cod may remain inshore for several years before adopting the migratory behaviour of mature cod around age 4 (Lear and Green 1984; Rose 1993).

Existing data on population structure within the northern cod are equivocal. On the one hand, geographic surveys

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(Hutchings et al. 1993), vertebral data (Templeman 1981; Lear and Wells 1984), and tag recovery data (Templeman 1974, 1979; Lear 1984; reviewed in Lear and Green 1984 and Taggart et al. 1995) all suggest that northern cod are divided into several distinct offshore spawning units; on the other hand, none of these observations constitute evidence of reproductive isolation among spawning units. Cod-tagging studies have provided evidence of population fidelity to particular overwintering–spawning areas on offshore banks and in some inshore bays, as well as evidence of vagrant movements among these areas (Lear 1984; Taggart et al. 1995; Wroblewski et al. 1996). However, tag recovery data yield no direct evidence of the extent of gene flow among spawning units.

In theory, allele frequency data from neutral genetic markers may provide the most stringent test of population differentiation. Thus far, however, evidence of population structure gained from genetic studies of northern and other western Atlantic cod has been mixed, and subjected to various interpretations. Data from a variety of allozyme (Mork et al. 1985) and mitochondrial DNA (mtDNA) studies (Smith et al. 1989; Carr and Marshall 1991*a*, 1991*b*; Pepin and Carr 1993; Carr 1995; Carr et al. 1995) have not permitted rejection of the null hypothesis that no genetic differentiation exists among various samples drawn from northwest Atlantic populations. In contrast, highly significant differences among populations have been detected with a small number of protein loci (Jamieson 1975; Cross and Payne 1978), more recently with a suite of nuclear DNA restriction fragment length polymorphism (RFLP) loci (Pogson et al. 1995), and most recently with nuclear microsatellite loci (Ruzzante et al. 1996*a*).

Several authors have invoked selection (non-neutrality of genetic loci) as an explanation for the contrast in the results obtained with different genetic markers, but differ in their view of the direction taken by selective forces. Some (e.g., Mork and Sundnes 1985; Mork et al. 1985) have suggested that selection is acting to increase heterogeneity at some loci (thereby exaggerating the distinctiveness of populations), whereas Pogson et al. (1995) have argued that balancing selection may be acting to homogenize variation expressed by some protein loci across different locales (thereby underrepresenting the isolation of populations).

Notwithstanding the possible role of selection, loci that exhibit significant geographic variation in cod have tended to exhibit moderate to high levels of polymorphism, whereas those that do not have tended to be less polymorphic, with low heterozygosities and (or) low frequency of variant alleles or haplotypes (Jamieson 1975; Cross and Payne 1978; Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991*a*; Pepin and Carr 1993; Carr 1995; Carr et al. 1995; Pogson et al. 1995). This would suggest that studies of cod population structure at less than ocean basin scales might be more informative if highly polymorphic, and preferably neutral, markers are used.

We have initiated such studies utilizing highly polymorphic microsatellite markers. The attributes of microsatellites as genetic markers and their application to fisheries have been reviewed elsewhere (Park and Moran 1994; Wright and Bentzen 1994; O'Reilly and Wright 1995). Briefly, microsatellites are repetitive arrays of di-, tri-, or tetra-nucleotide sequences in the nuclear genome. They exhibit high levels of allelic polymorphism in repeat number, are presumed to be selectively neutral (but see Warren 1996), and are readily assayed from small amounts of fresh or preserved tissue using the polymerase chain reaction (PCR). Recent studies employing microsatellites have detected high levels of genetic variation in cod (Brooker et al. 1994; Ruzzante et al. 1996*a*), as well as genetic differences among some moderately distant locations in the western Atlantic (S.K. McConnell, Department of Biology, Dalhousie University, Halifax, N.S., personal communication) and even between northern cod aggregations overwintering inshore and offshore spawning aggregations (Ruzzante et al. 1996*a*).

Here we report on polymorphism at six microsatellite loci in northern cod sampled from offshore overwintering–spawning locations spanning their range from the northern Grand Bank northwest to Hamilton Bank off Labrador, as well as from two other northwest Atlantic locations, Flemish Cap and the Scotian Shelf, and from one northeast Atlantic location in the Barents Sea. We report results that lead us to reject the null hypothesis of no genetic differentiation among cod from various northwest Atlantic regions, and hence to conclude that northwest Atlantic cod belong to multiple, genetically distinguishable populations. Further, we describe evidence that northern cod may comprise more than one population. We also use the results of our study to address questions concerning the relative utility of microsatellites as genetic markers for cod, and the various approaches that can be taken in analyzing microsatellite data.

Materials and methods

Sample collections

Northern cod were collected during January and February of 1992 and 1993 from nine different sample populations stretching from Hamilton Bank in the north to the "Nose" of the Grand Bank in the south (Fig. 1, Table 1). These samples were collected from prespawning aggregations using an otter trawl fishing at depths between 300 and 500 m. Tissue samples of cod from the Flemish Cap (Fig. 1, Table 1) were secured in the summer of 1993 from the commercial trawl fishery bycatch (nominal depths of 30 m) and were collected by onboard fisheries observers retained by the Canada Department of Fisheries and Oceans. Samples from the Scotian Shelf (Fig. 1, Table 1) comprised recently hatched cod larvae collected within a gyrelike water mass on Western Bank in November of 1992 (Taggart et al. 1996; Ruzzante et al. 1996*b*). Tissue samples of Barents Sea cod were taken from frozen cod heads that were off-loaded from a Russian freezer trawler and were provided by National Sea Products Ltd., Arnolds Cove, Newfoundland, in July 1993 (lot No. 686). The fish had been captured some time during the first half of 1993 at an unknown location in the Barents Sea (International Council for the Exploration of the Seas (ICES) area I).

The average fork lengths of cod were highly variable among samples (Table 1) and (excluding the larval cod sample) ranged from 30 cm (Nose of the Grand Bank in 1993) to 60 cm (Flemish Cap in 1993). The estimated ages of these cod (derived from length at age relationships) were equally variable (Table 1) and ranged from 2 to 6 years. The sex ratio of the analyzed samples was approximately 1:1 for samples where the sex of the individual was known, although the majority of samples were from immature or first time spawning fish (Table 1).

Sample tissues and genetic analyses

Blood and muscle from juvenile and mature cod and eye tissues from larval cod were used as the DNA source for genetic analysis. Blood samples were collected from live, or recently dead, cod using a sterile 2-mL hypodermic needle (21 gauge) inserted ventrally into the blood vessels that run in the haemal arches of the vertebrae between the anal fin and the caudal peduncle, and ~1 mL of blood was withdrawn and

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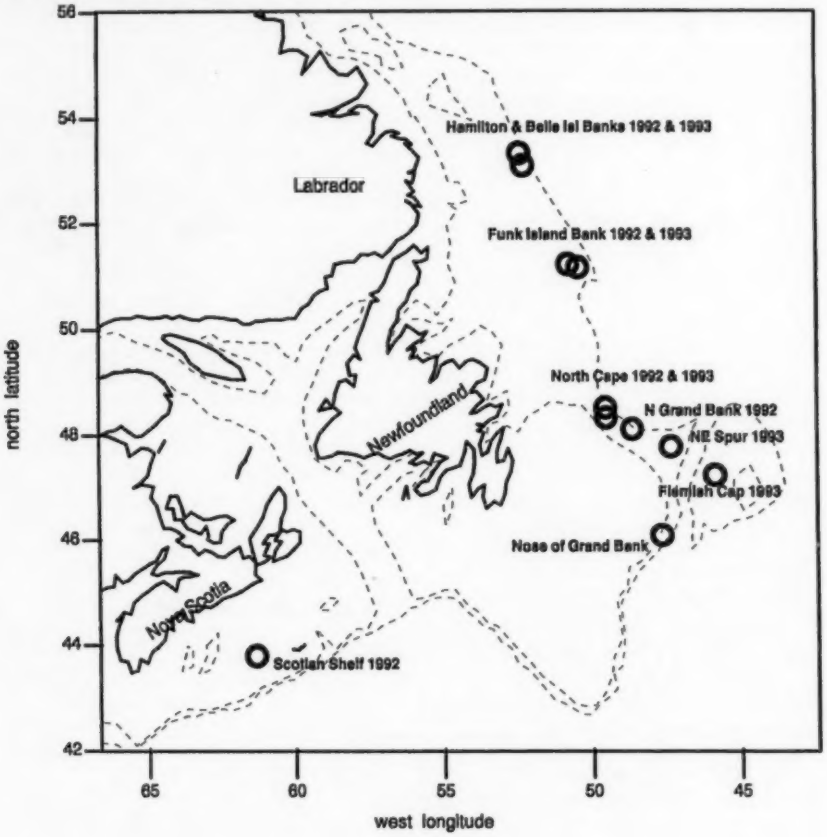
Fig. 1. Bathymetric chart (200- and 1000-m isobaths) of the northwest Atlantic shelf regions showing the dates and bank affiliations for the cod samples collected in 1992 and 1993 and used for microsatellite analyses.



preserved immediately in 5 mL of 95% ethanol and stored. Muscle tissue samples for genetic analysis were taken from the anterior of the tongue of dead cod and preserved in 95% ethanol. Larvae were preserved in liquid nitrogen immediately following collection at sea (Taggart et al. 1996). DNA extraction from alcohol-preserved tissue was as described in Bentzen et al. (1990) and Ruzzante et al. (1996*a*). DNA was released from eyeballs of larval cod as described in Ruzzante et al. (1996*b*).

PCR was carried out on 1–2 μL of purified or crude cod DNA as previously described by Brooker et al. (1994) using six sets of cod microsatellite primers: Gmo2, Gmo132, and Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), Gmo120 (Ruzzante et al. 1996*a*), and Gmo141 (Ruzzante et al. 1996*b*). Gmo2, Gmo4, Gmo120, Gmo132, and Gmo141 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_z$ repeat as defined by Tautz (1989). The PCR products were resolved on 6.5% denaturing polyacrylamide gels and the alleles sized relative to a sequence ladder generated from M13mp18.

Data analyses

Tests for departures from Hardy–Weinberg equilibrium (HWE) and deficiencies or excess of heterozygotes, and tests for heterogeneity in allele frequencies among samples were conducted using the χ^2 pseudoprobability programs CHIHW and CHIRXC (Zaykin and Pudovkin 1993). Both programs use randomization procedures to estimate the significance of the χ^2 statistic (Roff and Bentzen 1989). In all cases, 1000 randomizations were used to estimate probability (*P*) values.

Genetic distances among population samples were estimated using Rogers' (Rogers 1972), allele sharing (Bowcock et al. 1994), average squared (Goldstein et al. 1995*a*), and $(\delta\mu)^2$ (Goldstein et al. 1995*b*) measures. Estimates of subpopulation structure were obtained using F_{ST} following Weir and Cockerham (1984) and the related R_{st} measure recently proposed for microsatellite data by Slatkin (1995). Significance values for estimates of genetic distance and subpopulation structure were determined as described in Ruzzante et al. (1996*b*) using Monte Carlo simulations and the bootstrap method (minimum 1000 resampling trials per individual comparison; Manly 1991), and critical significance levels for simultaneous statistical tests were assessed using sequential Bonferroni adjustments as outlined in Rice (1989).

The genetic relationships among cod populations were evaluated using the various genetic distance measures listed above as inputs for neighbour-joining and Fitch–Margoliash trees constructed using programs in the PHYLIP software package (version 3.5C; Felsenstein 1994). As these two methods of tree construction yielded the same topologies only the Fitch–Margoliash trees are presented here.

Results

Variability of microsatellite loci within and among populations

All six microsatellite loci surveyed were highly polymorphic

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Table 1. Cod sample collection statistics.

Collection location and date	*N*	Mean location[a]		Length (cm)		Estimated age (years)		Number mature
		Latitude	Longitude	Range	Mean	Range	Mean	
Hamilton and Belle Isle banks, 1992	8	53.31	–52.38	25–48	33.4	1.7–4.4	2.7	3
Funk Island Bank, 1992	30	51.25	–50.51	28–51	41.0	2.0–4.8	3.5	12
North Cape, 1992	84	48.49	–49.46	40–75	53.9	3.4–8.2	5.2	21
Northern Grand Bank, 1992	56	48.11	–48.50	25–63	40.0	1.7–6.4	3.4	20
Belle Isle Bank, 1993	30	53.11	–52.17	27–43	34.9	1.9–3.7	2.8	16
Funk Island Bank, 1993	60	51.22	–50.76	21–48	33.6	1.4–4.4	2.7	14
North Cape, 1993	30	48.4	–49.5	21–47	34.3	1.4–2.4	2.8	7
Northeast Spur, 1993	30	47.79	–47.26	23–58	32.0	1.5–5.7	2.5	4
Nose of Grand Bank, 1993	30	46.09	–47.59	23–69	30.1	1.5–7.3	2.3	1
Barents Sea, 1993	100	—	—	43–82	57.0	3.0–9.0	5.2	—
Flemish Cap, 1993	51	47.25	–45.75	38–94	60.1	3.1–11.4	6.1	3
Scotian Shelf, 1992	193	43.75	–61.35	Larvae	—	<30 days	—	0

[a]Decimal degrees.

in all cod populations. The number of alleles observed per locus ranged from 24 for Gmo132 to 58 for Gmo141 (40.8 ± 14.9 (mean ± SD); Table 2). Observed heterozygosities (H_o) were correspondingly high, ranging from 0.369 for Gmo132 in the Barents Sea sample to 1.0 for Gmo4, Gmo120, Gmo141, and Gmo145 in several populations (mean = 0.86; Table 3). In all cases variation among the microsatellite alleles occurred in size increments consisting of multiples of two base pairs (bp).

We pooled some samples of northern cod to facilitate further statistical analyses, including broader geographic comparisons. Pairwise comparisons using χ^2 pseudoprobability tests were made first across sampling years at the same location, and then among geographically related sampling areas. No significant differences in allele frequencies were found among any of the six microsatellites among the 1992 and 1993 samples from Hamilton, Belle Isle, and Funk Island banks; hence, these samples were pooled to form a single sample (hereafter, NORTH). Similar analyses revealed no significant differences among the 1992 and 1993 samples from the North Cape, Grand Bank, and Nose of the Bank, so these samples were combined to form a single pooled sample (SOUTH). A sample from the northeast Spur area located between the northern Grand Bank and the Flemish Cap did differ from SOUTH in allele frequencies for Gmo141 ($P = 0.005$; Table 4) and consequently was excluded from the pooled SOUTH sample.

Pseudoprobability tests revealed no significant departures from HWE for the pooled NORTH, pooled SOUTH, or Scotian Shelf samples (Table 3). Pseudoprobability tests also indicated that measures of heterozygote excess or deficiency (D) did not deviate significantly from zero for five of the microsatellites in all populations. The exception was Gmo141, for which D was negative (indicating a deficiency of heterozygotes) in five of six population samples; four of these departures were significant (Table 3).

Geographic variation in alleles: size and frequency

The size-frequency distributions of microsatellite alleles varied among NORTH, SOUTH, and the four unpooled samples (Table 3, Fig. 2). Variation in mean allele size was significant (Kruskal–Wallis test, $P \leq 0.006$) for all loci except Gmo2 among the six populations (Tables 2, 3).

Pairwise pseudoprobability tests revealed significant differences in allele frequencies at one or more loci for each pair of populations, with the exception of the NORTH versus northeast Spur comparison (Table 4). Pairwise comparisons involving the Barents Sea sample were most likely to be significant: 16 of 30 comparisons involving this population revealed significant differences in allele frequencies. With one exception, Gmo120, all six microsatellites exhibited significant heterogeneity in allele frequencies in at least some pairwise comparisons.

Table 2. Single locus statistics for cod microsatellites.

Locus	*N*	*n*	H_e	*P*
Gmo2	664	25	0.803	0.123
Gmo4	646	57	0.957	<0.001*
Gmo120	622	44	0.953	0.006*
Gmo132	672	24	0.769	<0.001*
Gmo141	640	58	0.97	<0.001*
Gmo145	647	37	0.938	<0.001*
Mean	648.5	40.8	0.898	—

Note: *N*, number of individuals; *n*, number of alleles; H_e, expected heterozygosity; *P*, probability that mean allele size is identical among NORTH, SOUTH, northeast Spur, Flemish Cap, Scotian Shelf, and Barents Sea samples, determined using Kruskal–Wallis tests (5 df). Tests considered significant following adjustment for six simultaneous tests ($\alpha = 0.008$) are indicated with an asterisk.

Broad-scale geographic variation was strongest for Gmo132 (Fig. 2). Although the allele distributions for NORTH, SOUTH, and northeast Spur were very similar ($P \geq 0.133$, Table 4), they differed sharply from those seen in other populations. In the three northern cod samples (north, south, and northeast Spur) the allele distribution for Gmo132 consisted of a single mode dominated by two alleles of 111 and 117 bp at 24–28 and 38–44% frequency, respectively. In contrast, the frequencies of the 111- and 117-bp alleles were 76 and 5% in the Barents Sea sample, and 55 and 19% in the Flemish Cap sample. The Scotian Shelf sample exhibited multiple modes of alleles at this locus, separated from each other by intervals of approximately 20 bp. One of these modes, dominated by 111- and 117-bp alleles, corresponded to the only mode seen in the other populations. The shape of this mode was similar to that seen in the northern cod samples. The two modal allele groups unique to the Scotian Shelf sample comprised alleles close in size to 137 and 157 bp, respectively, with a collective frequency of 22.1%.

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Table 3. Summary statistics for genetic variation in Atlantic cod population samples.

	Northern cod					
	Northeast Spur	NORTH	SOUTH	Scotian Shelf	Flemish Cap	Barents Sea
			Gmo2			
N	28	119	195	193	50	79
n	7	18	20	20	12	17
Allele size	110.83	111.90	112.54	111.91	112.14	113.39
H_o	0.679	0.798	0.749	0.725	0.92	0.759
H_e	0.764	0.794	0.805	0.773	0.827	0.842
D	–0.112	0.005	–0.07	–0.062	0.113	–0.097
P(HW)	—	0.386	0.757	0.681	—	—
P(D)	0.345	0.797	0.031	0.074	0.11	0.037
			Gmo4			
N	28	116	187	191	44	80
n	2	37	40	40	24	31
Allele size	205.46	208.32	212.63	209.88	203.42	211.18
H_o	0.929	0.966	0.925	0.958	0.864	0.963
H_e	0.926	0.955	0.953	0.957	0.938	0.939
D	0.003	0.011	–0.03	0.001	–0.079	0.026
P(HW)	—	0.985	0.98	0.977	—	—
P(D)	0.763	0.686	0.021	1	0.022	0.495
			Gmo120			
N	30	108	176	192	44	72
n	20	28	36	32	26	32
Allele size	189.55	188.47	189.85	187.67	191.72	187.51
H_o	0.967	0.972	0.92	0.938	0.841	0.972
H_e	0.934	0.946	0.95	0.953	0.938	0.95
D	0.035	0.028	–0.031	–0.016	–0.104	0.023
P(HW)	—	0.985	0.98	0.977	—	—
P(D)	0.729	0.686	0.021	1	0.022	0.495
			Gmo132			
N	30	118	197	193	50	84
n	7	10	12	18	8	10
Allele size	115.325	114.887	114.912	120.434	113.660	111.858
H_o	0.767	0.729	0.766	0.824	0.62	0.369
H_e	0.746	0.723	0.751	0.797	0.646	0.414
D	0.028	0.008	0.021	0.033	–0.041	–0.109
P(HW)	—	0.356	0.116	0.341	—	—
P(D)	0.818	0.915	0.646	0.322	0.669	0.127
			Gmo141			
N	28	114	194	193	32	79
n	26	46	47	53	28	39
Allele size	168.05	174.26	172.44	172.91	173.15	181.16
H_o	0.75	0.93	0.897	0.933	0.844	0.975
H_e	0.947	0.966	0.967	0.967	0.952	0.96
D	–0.208	–0.037	–0.073	–0.036	–0.114	0.015
P(HW)	—	0.972	0.972	0.997	—	—
P(D)	<0.001*	0.021	<0.001*	0.009*	0.003*	0.617
			Gmo145			
N	28	118	191	189	47	74
n	19	25	31	31	21	22
Allele size	180.80	176.79	175.31	176.23	176.17	174.03
H_o	0.964	0.932	0.916	0.937	0.957	0.851
H_e	0.928	0.932	0.94	0.934	0.921	0.921
D	0.039	0.001	–0.025	0.002	0.04	–0.075
P(HW)	—	0.995	0.978	0.992	—	—
P(D)	0.591	1	0.152	0.88	0.464	0.011
Mean H_e	0.874	0.886	0.894	0.897	0.87	0.838
SE	0.038	0.042	0.038	0.036	0.049	0.086

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Table 3 (*concluded*).

	Northern cod					
	Northeast Spur	NORTH	SOUTH	Scotian Shelf	Flemish Cap	Barents Sea
Mean H_o	0.842	0.888	0.862	0.886	0.841	0.815
SE	0.051	0.041	0.033	0.038	0.048	0.096
Mean D	–0.036	0.003	–0.035	–0.013	–0.031	–0.036
SE	0.041	0.009	0.014	0.014	0.014	0.026

Note: N, number of individuals; n, number of alleles; H_o, observed number of heterozygotes; H_e, expected number of heterozygotes; D, measure of heterozygote deficiency or excess ($(H_o - H_e)/H_e$); P(HW) and P(D), estimated probability levels based on pseudoprobability tests. Allele sizes are given as the mean. Tests considered significant following sequential Bonferroni adjustment for six tests per locus (initial $\alpha = 0.008$) are indicated with an asterisk (*).

Table 4. Probability of homogeneity of allele frequencies estimated from pairwise pseudoprobability tests.

Comparison		Gmo2	Gmo4	Gmo120	Gmo132	Gmo141	Gmo145
NORTH	SOUTH	0.552	<0.001*	0.443	0.643	0.195	0.214
NORTH	Northeast Spur	0.923	0.311	0.479	0.133	0.057	0.469
NORTH	Scotian	0.57	0.186	0.501	<0.001*	0.923	0.238
NORTH	Flemish	0.455	0.21	0.71	<0.001*	0.111	0.327
NORTH	Barents	0.002*	0.183	0.058	<0.001*	0.073	<0.001*
SOUTH	Northeast Spur	0.908	0.049	0.787	0.22	0.005*	0.321
SOUTH	Scotian	0.352	0.017	0.107	<0.001*	0.121	0.311
SOUTH	Flemish	0.229	<0.001*	0.269	<0.001*	0.032	0.789
SOUTH	Barents	<0.001*	<0.001*	0.024	<0.001*	<0.001*	0.001*
Northeast Spur	Scotian	0.822	0.217	0.208	0.066	0.017	0.323
Northeast Spur	Flemish	0.533	0.184	0.775	0.023	<0.001*	0.651
Northeast Spur	Barents	0.011	0.642	0.404	<0.001*	0.001*	0.001*
Scotian	Flemish	0.123	0.058	0.035	<0.001*	0.142	0.483
Scotian	Barents	<0.001*	0.001*	0.262	<0.001*	0.018	0.006
Flemish	Barents	0.707	0.012	0.405	<0.001*	0.002*	0.005

Note: Probability estimates considered significant following sequential Bonferroni adjustments for 15 simultaneous tests per locus (initial $\alpha = 0.003$) are indicated with an asterisk (*).

The heterogeneity of Gmo132 allele distributions across the range of populations examined was apparent in the results of the pairwise pseudoprobability tests (Table 4). Among a total of 15 pairwise comparisons between populations, 10 tests were significant for Gmo132, whereas the number of significant results obtained with the other five loci ranged from 0 to 5 (mean = 3).

Temporal variability

The majority (67%) of the cod in the northeast Spur sample were estimated to be from the 1990 year-class (age 2+ in 1993), and most of the remaining fish were from the 1989 and 1991 year-classes (17 and 10%, respectively; Fig. 3). In contrast, cod in the pooled SOUTH sample were broadly distributed among year-classes 1985–1990 (mean age = 5+ in 1993), with small contributions from 1991 and pre-1985 year-classes as well. Finally, we estimated that the pooled NORTH sample was composed primarily of the three year-classes 1988, 1989, and 1990 (17, 32, and 35, respectively), along with a small number of 1986, 1987, and 1991 fish.

We used χ^2 pseudoprobability tests to evaluate whether variation in allele frequencies among year-classes might confound the interpretation of genetic variation among geographic samples. Although the estimated year-class compositions of the northeast Spur, SOUTH, and NORTH samples differed from each other (Fig. 3), heterogeneity of allele frequencies among age-classes was not significant for any of the six microsatellite loci in a pooled sample of SOUTH and northeast Spur fish (adjusted $\alpha = 0.008$, $P \geq 0.027$). Likewise, allele frequencies did not vary significantly for any of the loci among the 1989 and 1990 year-classes of cod in the NORTH sample ($P \geq 0.143$).

To further evaluate whether temporal variability of allele frequencies was a factor in our results, we split the NORTH and SOUTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 pseudoprobability tests of allelic heterogeneity for Gmo4, the locus that differed between NORTH and SOUTH. Pairwise comparisons revealed significant differences in allele frequencies for NORTH92 versus SOUTH92, NORTH92 versus SOUTH93, and NORTH93 versus SOUTH93, but not for NORTH92 versus NORTH93, NORTH93 versus SOUTH92, and SOUTH92 versus SOUTH93 (Table 5).

Estimates of genetic distance

When applied to our cod data, four measures of genetic distance (allele sharing, $(\delta\mu)^2$, Rogers', and average squared) yielded results that varied markedly in at least two ways (Table 6). First, the number of pairwise distances that were significantly greater than zero differed among the measures. Among 10 pairwise comparisons, all 10 genetic distances were significantly greater than zero for the $(\delta\mu)^2$ and allele sharing measures. For the Rogers' and average squared distances, the corresponding numbers of significant tests were nine and zero, respectively (Table 6). The second difference was apparent when we summarized the pairwise distances using Fitch–

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Fig. 2. Allele frequencies of microsatellite loci in cod populations. Shown are the absolute allele frequencies for SOUTH, and for other populations, the difference between the allele distribution in each population and the observed distribution in SOUTH (i.e., other-SOUTH). Absolute/relative frequencies and allele size in base pairs (bp) are given by the ordinate and abscissa, respectively, for each locus.

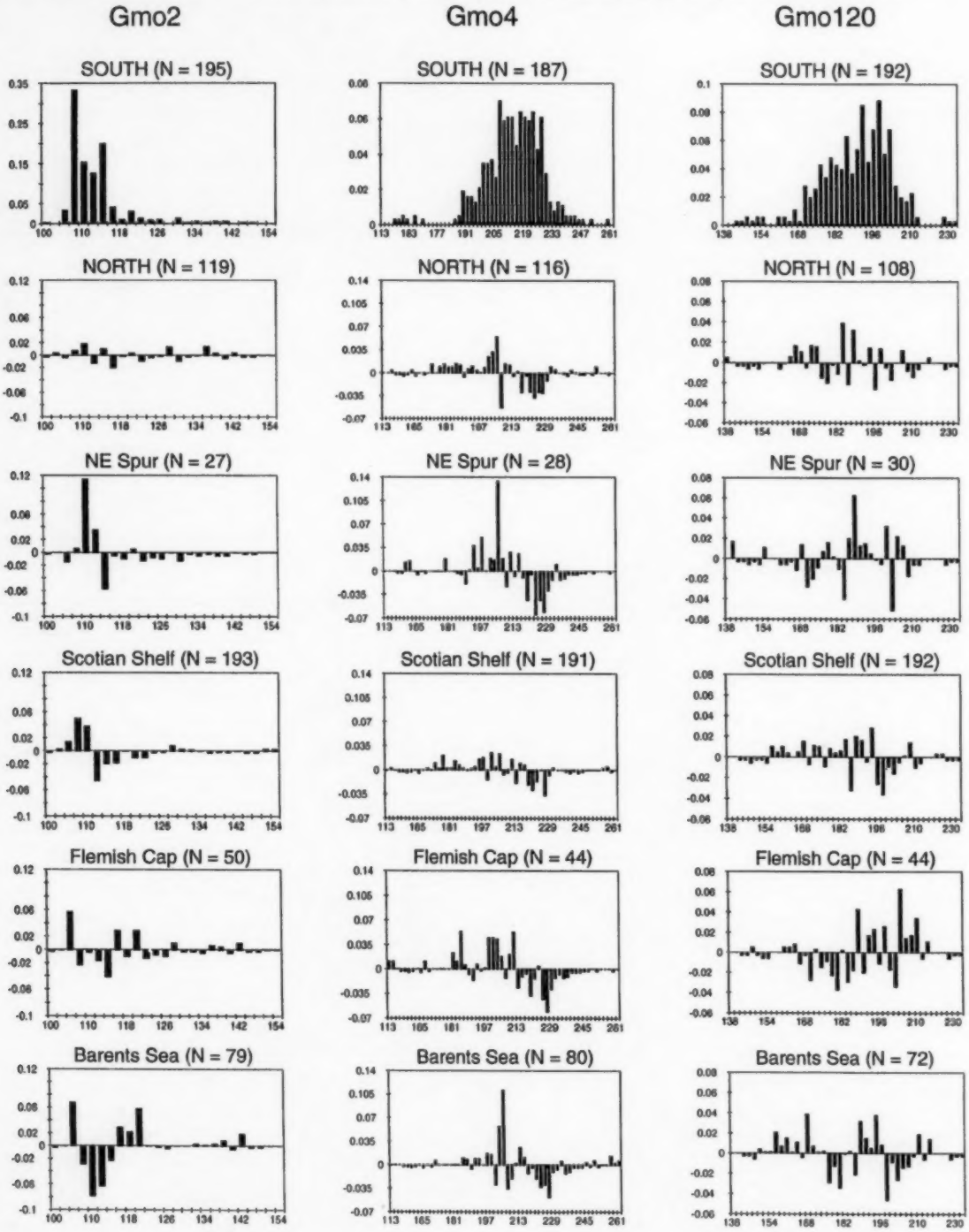
Gmo2
Gmo4
Gmo120
SOUTH (N = 195)
SOUTH (N = 187)
SOUTH (N = 192)
NORTH (N = 119)
NORTH (N = 116)
NORTH (N = 108)
NE Spur (N = 27)
NE Spur (N = 28)
NE Spur (N = 30)
Scotian Shelf (N = 193)
Scotian Shelf (N = 191)
Scotian Shelf (N = 192)
Flemish Cap (N = 50)
Flemish Cap (N = 44)
Flemish Cap (N = 44)
Barents Sea (N = 79)
Barents Sea (N = 80)
Barents Sea (N = 72)

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Fig. 2 (*concluded*).

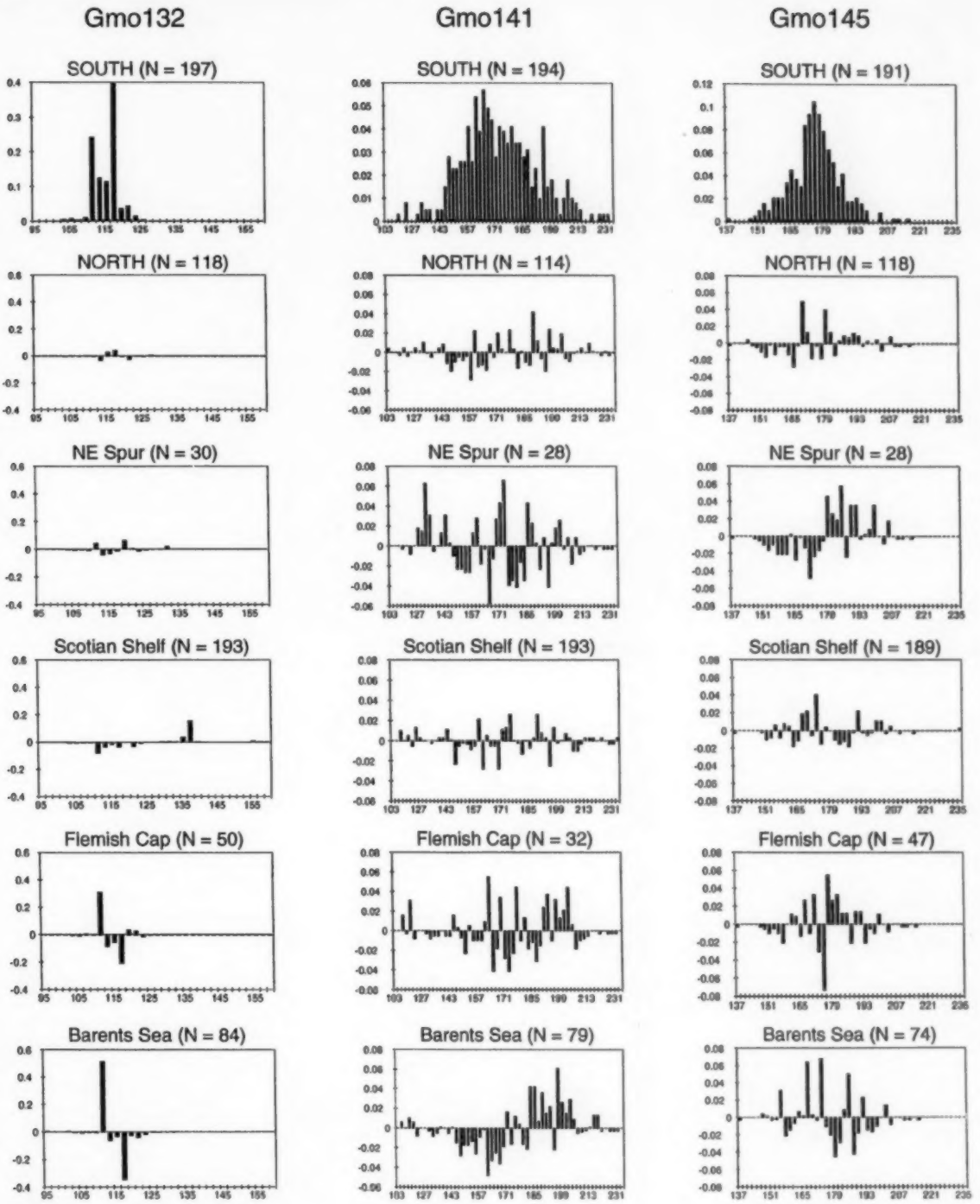
Gmo132
Gmo141
Gmo145
SOUTH (N = 197)
SOUTH (N = 194)
SOUTH (N = 191)
NORTH (N = 118)
NORTH (N = 114)
NORTH (N = 118)
NE Spur (N = 30)
NE Spur (N = 28)
NE Spur (N = 28)
Scotian Shelf (N = 193)
Scotian Shelf (N = 193)
Scotian Shelf (N = 189)
Flemish Cap (N = 50)
Flemish Cap (N = 32)
Flemish Cap (N = 47)
Barents Sea (N = 84)
Barents Sea (N = 79)
Barents Sea (N = 74)

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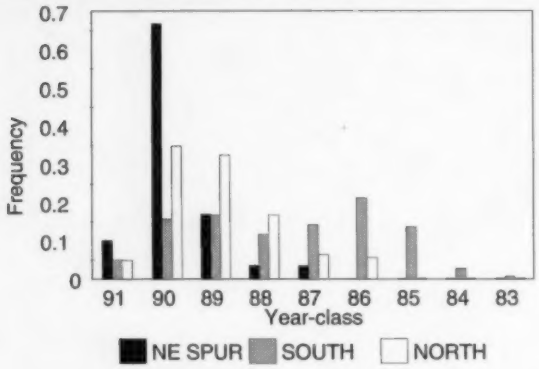
Fig. 3. Year-class contributions (distributions) for the northern cod samples as determined according to length at age estimates.



Margoliash trees. Both the $(\delta\mu)^2$ and the Rogers' tree aligned northwest Atlantic populations into two groups distinct from the relatively divergent Barents Sea sample (Figs. 4A and 4C, respectively). One group consisted of the NORTH, SOUTH, and Scotian Shelf samples, and the other consisted of the Flemish Cap sample. In contrast, the allele sharing tree presented little evidence of hierarchical population structure, with only a weak indication of a NORTH, SOUTH, Scotian Shelf cluster and no indication of divergence between the Barents Sea sample and the four western Atlantic samples (Fig. 4B). Finally, the average squared distance tree portrayed the four western and one eastern Atlantic population as an unresolved polytomy, with a single central node and terminal branches of similar length (Fig. 4D).

Estimates of population substructure

F_{ST} estimates calculated for six different populations ranged from 0.0 for Gmo120 to 0.092 for Gmo132 (Table 7). Except for Gmo2, all nonzero F_{ST} values were significant, as was the combined F_{ST} (= 0.015, $P < 0.001$). We found that excluding the small northeast Spur sample from the analysis had little overall effect (combined $F_{ST} = 0.016$, $P < 0.001$). F_{ST} values were larger between a pooled western Atlantic sample (NORTH, SOUTH, and Scotian Shelf, but excluding the Flemish Cap sample) and the Barents Sea ($F_{ST} = 0.037$, $P < 0.001$). On a finer geographic scale, F_{ST} values for a comparison between all northern cod samples and the Flemish Cap sample were only marginally smaller than in the larger scale six-population analysis, but only significant because of a large contribution from Gmo132 ($F_{ST} = 0.090$, $P < 0.001$). The overall F_{ST} between all northern cod and the Scotian Shelf sample was significant ($F_{ST} = 0.003$, $P < 0.001$) but also entirely dependent on Gmo132 ($F_{ST} = 0.021$, $P < 0.001$). Finally, the overall F_{ST} was significant for a comparison among samples in the NORTH group ($F_{ST} = 0.007$, $P = 0.002$), but not for a comparison among samples in the SOUTH group ($F_{ST} = 0.002$, $P = 0.080$) or between NORTH and SOUTH ($F_{ST} = 0.000$).

The R_{ST} analyses (Table 7) presented a more complex picture than that obtained with F_{ST}. In various analyses, the single-locus R_{ST} estimates ranged from large and positive to large and negative values for all loci except Gmo145. The most extreme example of this effect involved Gmo132, for which the estimated R_{ST} was 0.208 ($P < 0.001$) in a three-way comparison between NORTH, SOUTH, and Scotian Shelf but assumed a value of –0.131 when all northern cod were pooled into a single sample and compared with the Scotian Shelf sample.

Table 5. Probability of homogeneity of allele frequencies for Gmo4 estimated from pairwise pseudoprobability tests.

Comparison		*P*
NORTH92	NORTH93	0.116
NORTH92	SOUTH92	<0.001*
NORTH92	SOUTH93	0.004*
NORTH93	SOUTH92	0.188
NORTH93	SOUTH93	0.036*
SOUTH92	SOUTH93	0.707

Note: Probability (*P*) estimates considered significant are indicated with an asterisk (*).

In comparison to the results obtained with F_{ST}, fewer single- and multi-locus R_{ST} values were significantly greater than zero (Table 7). The overall R_{ST} was not significant in comparisons of six (mean $R_{ST} = 0.034$, $P = 0.060$) or five populations (excluding northeast Spur, mean $R_{ST} = 0.021$, $P = 0.126$) despite large positive (and highly significant) contributions from Gmo132 and Gmo145. The overall R_{ST} was also not significant in a comparison between pooled NORTH, SOUTH, and Scotian Shelf samples and the Barents Sea (mean $R_{ST} = 0.037$, $P = 0.119$). Overall R_{ST} values were negative and hence not significantly different from zero in comparisons between pooled northern cod and Flemish Cap, and between pooled northern cod and Scotian Shelf. By contrast, the overall R_{ST} was positive (= 0.018) and significant ($P = 0.003$) in a three-way comparison between NORTH, SOUTH, and Scotian Shelf, owing to contributions from Gmo132 ($R_{ST} = 0.208$, $P < 0.001$) and Gmo145 ($R_{ST} = 0.027$, $P = 0.015$). Single- and multi-locus R_{ST} values were not significant in other comparisons within NORTH or SOUTH, or between NORTH and SOUTH (Table 7).

Discussion

Our results lead us to reject the null hypothesis of no genetic differentiation among northern cod in particular, and northwest Atlantic cod in general, a result contrary to that obtained in previous studies employing less variable mtDNA or allozyme markers (Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991*a*, 1991*b*; Árnason et al. 1992; Carr 1995; Carr et al. 1995; but see Cross et al. 1978; Pogson et al. 1995). We employed a variety of measures to assess genetic differentiation and population structure in cod using the microsatellite data, and most, but not all, of the measures revealed significant genetic differences among population samples at large (ocean basin) and meso (regional bank) scales. Our results, therefore, have various implications for the population biology of cod, the management of cod stocks, the utility of microsatellites as genetic markers in population studies, and the statistical methods used to interpret the data. We consider these issues below.

Population structure: ocean basin scales

We observed the strongest differentiation between the Barents Sea sample and the northwest Atlantic samples, a feature seen in χ^2 tests of allele frequency homogeneity, $(\delta\mu)^2$, and Rogers'

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Table 6. Genetic distances (above diagonal for each section) and probability estimates (below diagonal for each section).

	NORTH	SOUTH	Flemish	Scotian	Barents
		$(\delta\mu)^2$			
NORTH		5.37	7.77	6.24	13.35
SOUTH	0.008*		17.85	7.78	18.62
Flemish	0.042*	<0.001*		16.85	27.54
Scotian	0.003*	<0.001*	<0.001*		29.03
Barents	<0.001*	<0.001*	<0.001*	<0.001*	
		Allele sharing			
NORTH		0.303	0.386	0.286	0.326
SOUTH	0.010*		0.460	0.307	0.322
Flemish	0.015*	<0.001*		0.448	0.383
Scotian	0.038*	<0.001*	<0.001*		0.344
Barents	0.004*	<0.001*	0.013	<0.001*	
		Rogers'			
NORTH		0.057	0.128	0.072	0.159
SOUTH	0.37		0.122	0.070	0.157
Flemish	<0.001*	<0.001*		0.138	0.126
Scotian	0.001*	<0.001*	<0.001*		0.159
Barents	<0.001*	<0.001*	<0.001*	<0.001*	
		Average squared			
NORTH		312.045	336.892	341.100	321.870
SOUTH	0.948		346.102	341.778	326.275
Flemish	0.445	0.277		373.290	357.642
Scotian	0.332	0.298	0.037		364.872
Barents	0.787	0.715	0.139	0.034	

Note: Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

genetic distances. This result is consistent with genetic differentiation of cod at Atlantic basin scales that has been previously documented using allozymes (Mork et al. 1985), mtDNA (Carr and Marshall 1991*a*, 1991*b*; Carr 1995), and nuclear RFLP markers (Pogson et al. 1995). Cod from the northwest Atlantic during the 1950s and 1960s, but not since 1966, have been known to stray to Greenland, Iceland, the North Sea, and the Barents Sea, but with extremely low and decreasing (from west to east) frequency (Taggart et al. 1995).

Mitochondrial DNA studies have revealed differing levels of genetic diversity across the eastern and western portions of the range of cod. Nucleon diversities (analogous to heterozygosity for nuclear loci) off Newfoundland and Nova Scotia are lower (18–38%) than they are in the northeast Atlantic (82–85%), a pattern that suggests that cod in the northwest Atlantic have been subject to one or more population bottlenecks (Carr and Marshall 1991*a*, 1991*b*; Carr 1995; Carr et al. 1995). Such a loss of genetic variation in northwest Atlantic cod is not evident in the microsatellite loci: mean expected heterozygosity among all six loci in our northwest Atlantic samples was 88%, compared with 84% for the Barents Sea sample. Allozyme and cDNA loci similarly show no evidence of a genetic bottleneck in the northwest Atlantic (Mork et al. 1985; Pogson et al. 1995), implying that any bottleneck(s) that may have occurred only influenced genetic diversity in mtDNA.

Population structure: northwest Atlantic

Comparisons of single-locus allele frequencies, single- and multi-locus F_{ST} values, and $(\delta\mu)^2$, allele sharing, and Rogers' genetic distances revealed significant genetic differences among northwest Atlantic population samples. Flemish Cap, Scotian Shelf, and northern cod samples all differed significantly from each other. Each of these regions are separated from each other by wide and deep submarine trenches. Within the northern cod complex, χ^2, $(\delta\mu)^2$, and allele sharing analyses suggested the presence of at least two genetically distinguishable groups: one comprising our pooled NORTH samples (Hamilton, Belle Isle and Funk Island banks; NAFO divisions 2J, 3K) and the other consisting of our SOUTH samples from the northern Grand Bank area (North Cape, Grand Bank, and Nose of the Bank; NAFO division 3L). One other northern cod sample, northeast Spur (division 3L), also differed at one locus (Gmo141) from the adjacent SOUTH pool of northern cod samples but not from the NORTH pool; this result could be an artifact of the small sample size for this group (Table 3) and (or) allele scoring problems affecting this sample (see below).

The genetic variation we observed in northwest Atlantic cod could conceivably result from three different causes: (*i*) selective effects on non-neutral genetic markers, (*ii*) temporal or "chaotic" (*cf.* Hedgecock 1994) variation in allele frequencies among samples not associated with stable spatial differentiation, or (*iii*) divergence through the effects of genetic drift and mutation acting on reproductively isolated populations. We consider each in turn below.

We have no direct evidence of the neutrality or non-neutrality of the microsatellite markers employed in this survey, but since they all consist of dinucleotide repeats, they cannot be part of the protein-coding regions of genes. It is possible that they might be indirectly involved in selection through close linkage to a selectively important gene, but this is unlikely, at least statistically. Moreover, the extreme degree

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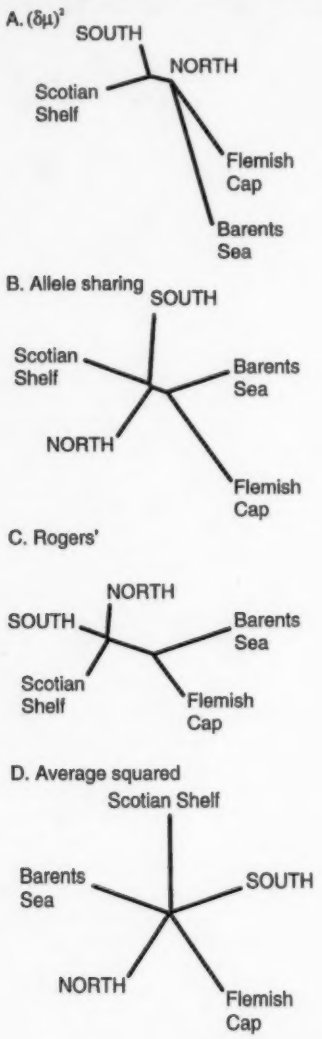
Fig. 4. Fitch–Margoliash phenograms depicting relationships among cod populations based on four measures of genetic distance.



of polymorphism exhibited by these microsatellites argues against fitness effects associated with particular alleles.

We evaluated the relative significance of temporal or chaotic variation versus stable geographic variation to our results in two ways. First, we conducted χ^2 tests of allele frequency heterogeneity among the inferred year-classes of cod within the NORTH and SOUTH (in this case including northeast Spur) pools of samples and we observed no significant variation among the year-classes for any of the six loci. Second, we split the SOUTH and NORTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 tests of allele frequency heterogeneity for Gmo4, the single locus that differed between the NORTH and SOUTH pools. Differences in allele frequency were significant for NORTH92 versus SOUTH92, NORTH92 versus SOUTH93, and NORTH93 versus SOUTH93 comparisons, but not significant for NORTH93 versus SOUTH92, NORTH92 versus NORTH93, and SOUTH92 versus SOUTH93 comparisons (Table 5). In general, these results are more consistent with the hypothesis that the difference seen with Gmo4 between the NORTH and SOUTH pools reflects stable spatial rather than temporal or chaotic variation among samples; however, the lack of significance in the NORTH93 versus SOUTH92 comparison leaves some uncertainty over this issue. The χ^2 results and direct inspection of the allele frequency data both suggest the following two points: (*i*) the SOUTH92 and SOUTH93 pooled samples were closely similar and (*ii*) the NORTH93 sample was somewhat more similar to the two SOUTH samples than was the NORTH92 sample. Clearly, the tentative conclusion that the difference between NORTH and SOUTH is a stable phenomenon needs independent testing using surveys of additional samples drawn from the areas and populations we have examined here.

Our data are not sufficient to permit a direct evaluation of the relative importance of spatial versus temporal variation for comparisons outside of northern cod. However, a number of independent surveys have confirmed the presence of the large (>131 bp) Gmo132 alleles in Scotian Shelf cod and their absence in northern cod (Brooker et al. 1994; Taggart 1995; Ruzzante et al. 1996*a*, 1996*b*), indicating that this difference between northern and Scotian Shelf cod is a stable phenomenon.

The genetic differentiation we observed among northwest Atlantic cod populations using microsatellite markers stands in contrast with the results of surveys of mtDNA sequence variation in northwest Atlantic cod (Carr and Marshall 1991*a*, 1991*b*; Pepin and Carr 1993; Carr 1995; Carr et al. 1995). Conversely, our microsatellite analyses show some similarities to studies of Cross and Payne (1978) and Pogson et al. (1995) that surveyed protein and nuclear RFLP loci, respectively. Both of these studies detected highly significant genetic differences among northwestern Atlantic populations of cod.

Cross and Payne (1978) studied variation at two loci, transferrin (*Tf*) and phosphoglucose isomerase (*PGI–1*), across a range of locations that included several that corresponded closely to the locations we sampled in the northwest Atlantic, but some 20 years earlier. In Cross and Payne's study, as in ours, significant genetic differences were detected between Flemish Cap, Scotian Shelf, and northern cod populations. Cross and Payne's results suggested a split between populations south of the Laurentian channel (including the Scotian Shelf) and those to the north. In our study, such a distinction is only weakly suggested by the $(\delta\mu)^2$ distances, and not by the other distance measures. However, as noted above, the Laurentian channel does mark a discontinuity in geographic variation at the Gmo132 locus, with alleles larger than 131 bp frequently observed south of the Laurentian channel, but never to the north (Fig. 3).

With respect to the three conceivable explanations outlined earlier for the genetic variation that we detected in our analyses of northwest Atlantic cod, and on the basis of the ensuing arguments, we conclude that (*iii*) divergence through the effects of genetic drift and mutation acting on reproductively isolated populations is the simplest and most consistent explanation.

Our results also show some similarity to those of Cross and Payne (1978) regarding relationships among populations

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Table 7. F_{ST} and R_{ST} values for cod microsatellite loci.

	1	2	3	4	5	6
			F_{ST}			
Gmo2	0.003 (0.030)	0.011 (0.005*)	0.000 (na)	0.000 (na)	0.001 (0.130)	–0.002 (na)
Gmo4	0.003 (<0.001*)	0.005 (0.001*)	0.002 (0.132)	0.002 (0.003*)	0.000 (na)	0.004 (0.004*)
Gmo120	0.000 (na)	0.000 (na)	0.001 (0.318)	0.000 (na)	0.000 (na)	0.000 (na)
Gmo132	0.092 (<0.001*)	0.200 (<0.001*)	0.090 (<0.001*)	0.015 (<0.001*)	0.021 (<0.001*)	–0.001 (na)
Gmo141	0.003 (0.004*)	0.004 (0.001*)	0.002 (0.139)	0.000 (na)	0.000 (na)	0.000 (na)
Gmo145	0.003 (0.004*)	0.007 (<0.001*)	0.002 (0.177)	0.006 (0.179)	0.000 (na)	0.000 (na)
Overall	0.015 (<0.001*)	0.037 (<0.001*)	0.014 (<0.001*)	0.003 (<0.001*)	0.003 (<0.001*)	0.000 (na)
			R_{ST}			
Gmo2	0.102 (0.137)	–0.129 (na)	–0.057 (na)	0.014 (0.408)	–0.061 (na)	–0.006 (na)
Gmo4	0.026 (0.368)	0.085 (0.142)	–0.117 (na)	0.008 (0.296)	–0.003 (na)	0.006 (0.385)
Gmo120	0.0112 (0.391)	–0.090 (na)	0.001 (0.496)	0.016 (0.137)	0.004 (0.407)	0.025 (0.093)
Gmo132	0.415 (<0.001*)	0.387 (<0.001*)	–0.034 (na)	0.208 (<0.001*)	–0.131 (na)	0.013 (0.207)
Gmo141	–0.017 (na)	0.039 (0.233)	–0.075 (na)	–0.002 (na)	–0.013 (na)	–0.012 (na)
Gmo145	0.098 (0.002*)	0.049 (0.216)	0.117 (0.024)	0.027 (0.015)	–0.012 (na)	0.037 (0.009)
Overall	0.034 (0.060)	0.037 (0.119)	–0.051 (na)	F0.018 (0.003*)	–0.016 (na)	0.005 (0.319)

Note: *P* values are in parentheses. 1, among six populations (NORTH, SOUTH, Flemish Cap, Scotian, Barents, northeast spur); 2, between all northern cod and Scotian Shelf (pooled) and Barents Sea; 3, between all northern cod (pooled) and Flemish Cap 94; 4, between NORTH, SOUTH, and Scotian Shelf; 5, between all northern cod (pooled) and Scotian Shelf; 6, between NORTH and SOUTH. Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

within the northern cod complex. Their results, like ours, suggest a subdivision between cod from the northeastern Newfoundland Shelf (corresponding to our NORTH pool) and cod from the more southerly Grand Bank areas. Their study, however, included samples from the southern Grand Bank. Moreover, cod in Cross and Payne's study that came from our SOUTH area (North Cape and northern Grand Bank) showed mixed affinities between the more northerly and southerly groups.

The genetic differentiation between northern cod, Flemish Cap, and Scotian Shelf populations suggests that the deep ocean trenches that separate these populations act as barriers to gene flow. A similar conclusion was reached for haddock (*Melanogrammus aeglefinus*) in the western Atlantic by Zwanenburg et al. (1992) on the basis of mtDNA data.

The isolating effect of ocean channels or other hydrographic features appears most extreme for the Flemish Cap. In both our study and that of Cross and Payne, Flemish Cap cod were more divergent from geographically proximate Grand Bank samples than the latter were from more spatially distant Scotian Shelf and Labrador samples. Cross and Payne (1978) speculated that the distinctiveness of Flemish Cap cod might result from their separate descent from an isolated glacial refuge around Flemish Cap. Regardless of the historical and hydrographic factors involved, the genetic distinctiveness of the Flemish Cap cod demonstrate that genetic differentiation of cod populations can occur on geographic scales of 200 km or less. The relative isolation of cod on the Flemish Cap is also supported by tagging studies that show that all but 4% of cod tags reported recaptured ($n = 132$) from a release ($n = 704$) on the Flemish Cap were reported from the Flemish Cap. In addition, of the tens of thousands of cod tagged and released over the last 40 years in the Newfoundland and Grand Banks region (excluding Flemish Cap), none (0%) of 4836 recaptures in the 1950s, 4 (0.03%) of 13 600 recaptures in the 1960s, and 4 (0.02%) of 17 620 recaptures in the 1970s and 1980s were reported as captured from the Flemish Cap (Taggart et al. 1995), despite a history of intensive fishing activity on the Cap.

Although our results are consistent with the existence of reproductively isolated populations of cod, they also indicate that the extent of genetic differentiation between populations is of low magnitude. Both direct inspection of allele frequency distributions (see Fig. 2) and F_{ST} and R_{ST} measures indicate that the proportion of total genetic variation occurring among populations is small: approximately 1.5 and 3% for range-wide estimates of F_{ST} and R_{ST}, respectively (Table 7, column 1). The low level of genetic differentiation in cod undoubtedly stems, at least in part, from gene flow (straying), but may also reflect the relatively recent origin of cod populations (Pogson et al. 1995) and (or) the effects of convergent mutations acting on the microsatellite loci (see below).

Utility of microsatellites as genetic markers

We observed an average of 40.8 alleles per locus in the six microsatellites that we surveyed in cod. Heterozygosities averaged 86% across all loci and population samples and reached 100% in some cases. This level of variation is doubly remarkable. First, as noted elsewhere, cod microsatellites appear to be unusually variable relative to comparable dinucleotide microsatellites in many other species (Brooker et al. 1994; see also O'Reilly and Wright 1995). Second, the variation exhibited by the cod microsatellites substantially exceeds that seen with most other genetic markers in cod, although it is comparable to that observed with a minisatellite locus assayed in cod using the PCR (Galvin et al. 1995). By contrast, average heterozygosity and the average number of alleles per locus for 10 polymorphic allozyme loci were 13% and 1.9, respectively, for a basin-scale survey of cod (Mork et al. 1985). In a similar study involving nuclear RFLP loci detected with cDNA probes, average heterozygosities and numbers of alleles per locus (36% and 4.3) were intermediate between the values for allozyme and microsatellite loci (Pogson et al. 1995).

Our microsatellite survey as well as that of Ruzzante et al. (1996*a*) of cod microsatellite loci detected genetic differentiation in northwest Atlantic cod populations not evident with less

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variable genetic markers. These results suggest that hypervariable microsatellite markers offer some advantages of sensitivity in contexts where genetic variation exhibited by other genetic markers is low, or where genetic differentiation, at least in terms of neutral markers, is marginal. In other circumstances, however, hypervariability may be suboptimal for at least two reasons. (*i*) Large numbers of alleles create statistical problems in the form of large sampling errors. We countered this problem by pooling some samples, at the cost of forgoing both serial replication and analysis on some spatial scales for the northern cod complex (e.g., among the multiple marine banks encompassed within the NORTH pool). (*ii*) Hypervariability implies high mutation rates at these loci. The step-mutational process believed to prevail in microsatellites may result in many convergent or parallel mutations; hence, identity of allelic state does not necessarily denote identity by descent (Di Rienzo et al. 1994). In this regard, it is worth noting cases where the microsatellites did not reveal genetic differentiation. Although northwest Atlantic populations were individually distinguishable from the Barents Sea sample at two to five loci, only one microsatellite locus, Gmo132, distinguished all northwest Atlantic populations from the Barents sample. Since eastern and western Atlantic cod are known to be well differentiated at protein, nuclear RFLP, and mtDNA loci, the lack of consistent east–west differences in microsatellite loci could reflect the action of convergent mutations erasing the effects of genetic drift, and in a practical sense, it suggests that hypervariable microsatellites may not be the markers of choice for broad-scale surveys.

The least variable microsatellite, Gmo132, exhibited the greatest genetic differentiation in comparisons outside the northern cod complex. In the basin-scale comparisons of five populations (excluding northeast Spur), F_{ST} and R_{ST} values for Gmo132 were 45 and 37 times higher than the averages for the other five loci, respectively (Table 7). For the most part, basin-scale differences in the allele distributions of Gmo132 were due to marked differences in the frequencies of alleles common to multiple populations (Fig. 3), a pattern consistent with divergence primarily by genetic drift. One population, however, Scotian Shelf, exhibited a unique group of large alleles, indicating a role of (divergent) mutations in establishing genetic differentiation in this case. The unusual Gmo132 alleles present in the Scotian Shelf cod may have arisen through a mutational mechanism involving changes in the flanking sequence (D. Cook, unpublished observation) rather than within the repeat sequence, making this aspect of Gmo132 differentiation atypical.

Two complications that may attend the use of some microsatellite loci in population surveys are the occurrence of null alleles (alleles that fail to amplify) and the presence of artifactual "stutter" bands on gels that can make some loci difficult to score (both reviewed in O'Reilly and Wright 1995). Both phenomena can lead to scoring errors marked by an apparent deficiency of heterozygotes. We tested the inheritance of the microsatellite markers used in this study with captive families involving six parents, and the reproducibility of gel scoring with blind comparisons between two gel readers. The former test revealed no evidence of null alleles at any locus; the latter supported the reproducibility of gel scores (D. Cook, unpublished data). Nonetheless, one of the microsatellite loci we assayed, Gmo141, exhibited a significant deficiency of heterozygotes in four populations (see Table 3), suggesting the occurrence of null alleles and (or) scoring problems. Either problem could bias the results of allele frequency comparisons. In this regard, it may be noteworthy that the genetic difference that led to the exclusion of the northeast Spur sample from the SOUTH pool involved Gmo141; northeast Spur also showed the strongest heterozygote deficiency at this locus (Table 3).

For the reasons outlined above, the more moderately variable nuclear RFLP loci studied by Pogson et al. (1995) may present statistical and analytical advantages for population discrimination over the microsatellites employed in this study. On the other hand, the practical and economic advantages of PCR-based microsatellite assays over the Southern blot based RFLP technology are compelling (Wright and Bentzen 1994; O'Reilly and Wright 1995). Indeed, only PCR-based methods could allow the assay of the disparate tissue sources (including the larval cod eyeballs) used in this study.

The speed and modest cost of microsatellite analyses allowed us to achieve relatively large sample sizes in this study. This factor, coupled with differing levels of genetic variability, may account for the difference between our results and those obtained with mtDNA. The sample sizes in this study, which averaged 169 for NORTH, SOUTH, and Scotian Shelf, are at least 5 times greater than those reported to date for similar locations in the mtDNA studies (Carr 1995; Carr et al. 1995). Further, this study surveyed six (presumably) unlinked loci, whereas mtDNA is equivalent to a single locus. Finally, although the mtDNA studies have detected numerous haplotypes, the frequency distribution of these haplotypes around Newfoundland and Nova Scotia is characterized by a single haplotype that prevails everywhere at frequencies close to 85%, with individual variant haplotypes frequently observed only once, and invariably at ≤3% frequency (Carr and Marshall 1991*a*, 1991*b*; Carr 1995; Carr et al. 1995). Skewed haplotype distributions of this type may not offer the statistical power needed to detect the low (but significant) levels of population differentiation revealed with microsatellite and RFLP markers.

Statistical methods for analyzing microsatellite data

We employed a variety of analyses to reach the conclusions above. In general, familiar measures such as χ^2 contingency tests, F_{ST}, and Rogers' genetic distance not only proved to be sensitive indicators of genetic differentiation when evaluated using Monte Carlo methods, they also yielded similar results. The genetic distance measure, $(\delta\mu)^2$, recently proposed by Goldstein et al. (1995*b*) for microsatellites, also revealed significant genetic differentiation in a pattern largely congruent with the previous methods. Three other measures proposed specifically for microsatellite data gave variable results; these included two other genetic distance measures, allele sharing (Bowcock et al. 1994) and average squared (Goldstein et al. 1995*a*), as well as R_{ST}, an analog of F_{ST} (Slatkin 1995).

R_{ST}, like F_{ST}, estimates the proportion of total genetic variation that occurs between populations. Unlike F_{ST}, however, R_{ST} incorporates information about the size of microsatellite alleles. The inclusion of allele size is expected to make R_{ST} more informative (Slatkin 1995). However, variances of estimates of R_{ST} in pairwise comparisons can be relatively large when sample sizes differ, even when both samples are large. By contrast, variances on F_{ST} estimates appear to be much less

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affected by unequal sample sizes (D.E. Ruzzante, unpublished data). In this study, R_{ST} estimates were relatively variable in comparison to F_{ST} estimates (Table 7). The variability inherent in the R_{ST} estimate was most striking in the different results obtained for two analyses, one in which NORTH, SOUTH, and Scotian Shelf were compared, and another in which the northern cod complex was pooled as a single group and compared with Scotian Shelf. R_{ST} estimates for Gmo132 in these two analyses were 0.208 and –0.131, respectively (Table 7). In contrast, F_{ST} estimates varied little from each other (0.015 and 0.021, respectively) and were both statistically significant ($P < 0.001$, Table 7). A comparison of the R_{ST} and F_{ST} estimates from the present study with those from Ruzzante et al. (1996*a*) suggests that the two estimates may perform differently under different conditions such as number of populations considered, nature of the differences among populations, and degree of population subdivision. It appears, however, that for analyses of R_{ST} it may be important to equalize sample sizes (D.E. Ruzzante, unpublished data).

Two criteria, the number of pairwise distances significantly greater than zero and concordance with previous genetic data and zoogeographic factors, suggest that the $(\delta\mu)^2$ and Rogers' genetic distances were approximately equally effective in resolving relationships among cod populations, and that the average squared distance was not useful. The numbers of pairwise distances significantly greater than zero among 10 comparisons were 10, 10, 9, and 0 for the $(\delta\mu)^2$, allele sharing, Rogers', and average squared distances, respectively (Table 6). Further, the $(\delta\mu)^2$ and Rogers' trees were both in accord with previous protein, mtDNA, and nuclear RFLP analyses as well as geographic considerations in depicting the Barents Sea sample as most divergent, and the three populations from the northwest Atlantic continental shelf (NORTH, SOUTH, and Scotian Shelf), as closely related (Figs. 4A, 4C). This pattern was not evident in the allele sharing tree (Fig. 4B). It should be added that the allele sharing distance has the limitation that its expectation is greater than zero even for within-populaton comparisons. Finally, the average squared distance tree revealed no structure at all among cod populations (Fig. 4D).

The Rogers' tree was very similar to a maximum likelihood tree based on allele frequencies (data not shown). The latter approach to tree construction corresponds to a model in which populations diverge by genetic drift alone, and it is insensitive to the genealogical relationships of the alleles involved (Felsenstein 1994). In contrast, the $(\delta\mu)^2$ measure is based on differences in mean allele size between populations and hence is explicitly based on the step mutational model (Goldstein et al. 1995*b*). Hence, for these populations and loci, drift- and mutation-based models appear to work equally well in portraying reasonable relationships among populations.

Implications for northern cod

This study was motivated in large part by interest in the underlying population structure of the northern cod stock complex, which has undergone intense fishing pressure over the last four decades and has reached an all-time low in abundance (Taggart et al. 1995). It was recently suggested by deYoung and Rose (1993) that northern cod population structure is formed by extensive egg and larval drift, followed by opportunistic (and nonphilopatric) recruitment of juveniles to whatever adult assemblages they find themselves near. Consequently, deYoung and Rose predicted that population structure in northern cod is neither genetically based nor temporally stable, particularly following what they showed to be an apparent southward shift in abundance occurring after 1988. Our data, which include cod from a variety of year-classes, but a majority from after 1988, are inconsistent with both predictions. We have shown evidence for at least two genetically discernable groups within the northern cod complex, our NORTH and SOUTH pools that correspond to the 2J3K and 3L management divisions. A related microsatellite study demonstrated genetic differences between two overwintering cod aggregations within this complex in NAFO division 3L, one inshore and the other offshore (Ruzzante et al. 1996*a*). The differences occurred despite the fact that cod of both migratory types intermingle inshore during the summer as a result of the feeding migration by offshore cod. As noted above, these joint findings deserve independent testing with additional samples, and ideally with additional markers. Such work, now in progress at Dalhousie University, will serve to refine our knowledge of population structure within the northern cod complex. At present, however, the results reported here, those of Ruzzante et al. (1996*a*), and the previous work of Cross and Payne (1978) collectively support, with some weight, the conclusion that northern cod are not comprised of a single, panmictic assemblage, but rather are composed of genetically distinguishable subunits, each of which appear to be geographically affiliated with spawning area. This suggests that it may be wise to seriously reconsider the current (and historical) management scheme for northern cod, which presumes one stock distributed throughout the inshore and offshore regions stretching from Hamilton Bank to the Grand Bank through NAFO divisions 2J, 3K, and 3L (Taggart et al. 1994).

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Grey seal predation on the eastern Scotian Shelf: modelling the impact on Atlantic cod

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Abstract: We modelled trends in the predation mortality of the Atlantic cod (*Gadus morhua*) stock on the eastern Scotian Shelf, Canada, by grey seals (*Halichoerus grypus*) and the potential effects of this mortality on cod recruitment over the period 1970–1994. Two predation models were explored: one in which the fraction of cod in the diet was constant and another in which the fraction of cod was proportional to cod abundance. The number of grey seals in the study area, from the Gulf of St. Lawrence and Sable Island populations, increased from about 5000 in 1970 to 64 000 in 1994, while total food consumption increased from 10 000 t·year^{-1} in 1970 to 121 000 t·year^{-1}. Under the constant ration model, grey seals consumed about 15 400 t of cod in 1994 compared with about 6300 t under the proportional ration model. Grey seal predation mortality was only 10–20% of estimated fishing until the fishery was closed in 1993, indicating that grey seals were not a major factor in the recent collapse of this stock.

Résumé : Nous avons modélisé les tendances de la mortalité du stock de morue franche (*Gadus morhua*) de l'est de la plate-forme néo-écossaise (Canada) due à la prédation par le phoque gris (*Halichoerus grypus*), et les effets potentiels de cette mortalité sur le recrutement de la morue pendant la période 1970–1994. Deux modèles de la prédation ont été explorés : dans l'un, la proportion de morue dans l'alimentation des phoques était constante, tandis qu'elle variait en fonction de l'abondance des morues dans l'autre. Le nombre des phoques gris présents dans la zone d'étude, qui appartenaient aux populations du golfe du Saint-Laurent et de l'île de Sable, a grimpé, passant d'environ 5000 têtes en 1970 à 64 000 en 1994, tandis que la consommation totale de nourriture passait dans la même période de 10 000 à 121 000 t·an^{-1}. Dans le modèle à ration constante, les phoques gris consommaient environ 15 400 t de morue en 1994 contre environ 6300 t dans le modèle à ration proportionnelle. La mortalité due à la prédation par le phoque gris correspondait à seulement 10–20% des prises commerciales estimées jusqu'à la fermeture de la pêche en 1993, ce qui permet de penser que ce pinnipède ne représentait pas l'un des facteurs principaux du récent effondrement du stock de morue.
[Traduit par la Rédaction]

Introduction

Seals are abundant predators in many marine ecosystems and as K-selected species may play a role in determining community structure (Estes 1979). Predation by seals also has the potential to reduce the yield from commercially valuable fish species (e.g., Beddington et al. 1985; Gulland 1987; Wickens et al. 1992), although as Estes (1979) observed there is little empirical data about "the roles of marine mammals in marine communities beyond poorly supported trophic arguments concerning marine mammal/fishery conflicts." Since Estes' paper was published, a number of estimates of food consumption by seal populations have been produced (e.g., Ashwell-Erickson and Elsner 1981; Anonymous 1984; Lavigne et al.1985; Ryg and Øritsland 1990; Olesiuk 1993; Perez and McAlister 1993; Ugland et al. 1993; Hammond and Fedak 1994), but fewer studies (Anonymous 1988; Sparholt 1990; Overholtz et al. 1991; Ugland et al. 1993) have attempted to estimate predation mortality or the impact of seal predation on fish population dynamics. In this paper, we develop a model to estimate trends in the consumption and resultant predation mortality by grey seals on the eastern Scotian Shelf (ESS) population of Atlantic cod (*Gadus morhua*) over the period 1970–1994.

The ESS Atlantic cod stock is located in NAFO subdivisions 4Vs and 4W off Nova Scotia, Canada (Fig. 1). Commercial landings of this stock ranged between 40 000 and 80 000 t from 1958 to 1974 before declining to a low of only 10 000 t in 1977 as a result of reduced stock abundance following a period of overexploitation. Landings increased to a peak of 50 000 t in 1985 as the stock rapidly recovered after fishing effort was reduced. The abundance and landings of the ESS cod stock have declined since the mid-1980s, reaching the lowest recorded level and closure of the directed fishery in 1993 (Mohn and MacEachern 1994). The causes for this latest dramatic decline in ESS cod abundance are poorly understood, but are thought to include high fishing mortality, unfavorable oceanographic conditions for cod growth and survival, and increased grey seal predation mortality (Fanning et al. 1995). The grey seal population in eastern Canada, and particularly that on the Scotian Shelf, has continued to increase over the last decade and has recently reached the highest level observed since the first estimates were made more than 30 years ago (Mansfield and Beck 1977; Hammill et al. 1992; W.T. Stobo, Bedford Institute of Oceanography, Dartmouth, N.S., personal communication).

The grey seal is the most abundant pinniped species on the Scotian Shelf. The major grey seal breeding colonies in eastern Canada are located on Sable Island, in the western part of the study area (i.e., NAFO division 4W), and in the southern Gulf

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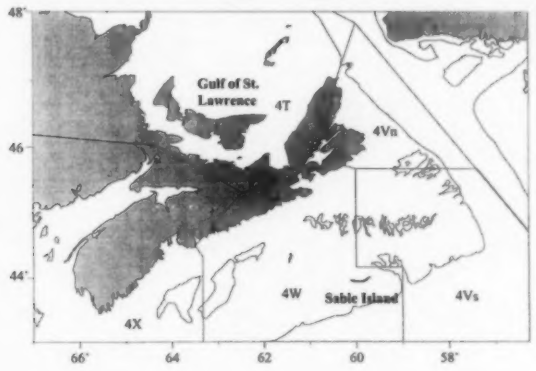
Fig. 1. Eastern Scotian Shelf (NAFO subareas 4W and 4Vs) and southern Gulf of St. Lawrence (NAFO subarea 4T).



of St. Lawrence (Fig. 1, Mansfield and Beck 1977). Both juveniles and adults from these breeding colonies are known to disperse over large areas of eastern Canada during the nonbreeding season, but large numbers are known to feed on the ESS throughout most of the year (Mansfield and Beck 1977; Stobo et al. 1990). Some exchange of adults occurs between the two main breeding colonies, but the degree of mixing is estimated to be small (Zwanenburg and Bowen 1990). Although the genetic structure of the eastern Canadian population is unknown, we consider grey seals from each of these two areas as representing separate populations.

The objectives of this paper are to model the population dynamics of grey seals; estimate trends in the age-specific consumption of ESS cod by grey seals; estimate trends in grey seal predation mortality on three size-classes of ESS cod; compare annual estimates of grey seal predation mortality with estimates of fishing mortality on ESS cod; examine the effect of grey seal predation mortality on yield per recruit, the temporal pattern of the stock-recruitment relationship, and potential future recruitment of ESS cod; and explore the sensitivity of the model output to uncertainty in the model variables and assumptions.

Seal population models

Zwanenburg and Bowen (1990) used a Leslie matrix to model the dynamics of the Gulf of St. Lawrence (Gulf) and Sable Island (Sable) grey seal populations in eastern Canada. They assumed that grey seals lived to a maximum age of 34 years and that males and females had the same natural mortality rates. We have built on the approach used by Zwanenburg and Bowen (1990) by constructing a deterministic, age-structured model, functionally similar to a Leslie matrix. The population numbers, $N[a;s;y]$, are described by a three-dimensional matrix having subscripts a, s, and y for age, sex (1 = male, 2 = female), and year, respectively. Thus, if the birth ogive is R, pup production in year y is

$$(1) \quad N[0;1;y] = N[0;2;y] = 0.5 \times \sum_{a} N[a;2;y] \times R[a]$$

In the present model, we used different rates of natural mortality in males and females after age 5 and different rates for the Gulf and Sable populations. Reduced male survivorship has been inferred on the basis of the oldest age of males and females observed in shot samples in Canada (Mansfield and Beck 1977) and the United Kingdom (Harwood and Prime 1978) and is consistent with natural mortality patterns observed in other sexually dimorphic pinnipeds (e.g., Lander 1981; LeBoeuf and Reiter 1988). The above data suggest that few grey seal males survive beyond age 25–30 years, whereas females survive to >40 years of age. To model the observed longevity of males, we increased the mortality rate of males aged 6 years and older by a factor of 3 (Table 1). This approach is similar to that advocated by Barlow and Boveng (1991) in that we scaled mortality by longevity and used different rates for pups, juveniles, and adults. In the model, no grey seal survives beyond 39 years of age.

Estimates of grey seal pup production and age-specific birth and mortality (including hunting mortality) rates from several sources (see below) were used as inputs to the population models. Separate parameters were estimated for the two populations because of differences in the observed rate of increase in pup production between the Gulf and Sable components and because the Gulf population was culled during the breeding season between 1967 and 1983. Pup production estimates for both the Gulf and Sable Island populations were fit using a population projection model with two parameters. The first parameter scaled the fixed pattern of natural mortality and the second scaled the estimates of total population size in 1967. The two-parameter population model was then optimized with a nonlinear least squares (NLLS) fit (Press et al. 1988). The population age distributions in 1967 were then updated by taking the NLLS-derived rates of natural mortality and finding a stable age distribution. This distribution was then used to fit the two parameters again until the whole procedure stabilized. The resultant age-specific estimates of survivorship for males and females in the two populations are shown in Table 1.

Mid- to late-term pregnancy rates from Zwanenburg and Bowen (1990) were used as birth rates in the models (Table 2). These data are from Mansfield and Beck (1977) except that the Sable Island rate for females aged 6 years and older was increased to 91 to generate the observed rate of increase in Sable Island pup production (see Zwanenburg and Bowen 1990). We assumed that the age-specific birth schedule of both populations was constant over the period 1970–1994. Recent data collected in the Gulf of St. Lawrence (Hammill and Gosslein 1995) are similar to the earlier data presented by Mansfield and Beck, lending support for this assumption. We also assume that the age-specific mortality rates of the Sable population have remained constant. While this cannot be strictly true, the exponential increase in pup production on Sable Island suggests that population parameters have been reasonably constant over the period of the simulation. In the case of the Gulf population, the mortality schedule clearly varied over time as a result of the cull on the breeding grounds between 1967 and 1984. In the model, these culls from the Gulf population were removed each year before natural mortality was applied.

Bootstrap estimates of confidence limits on population size

Bootstrapping (Efron 1982) is a computer intensive technique in which the underlying data or intermediate results are resampled,

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Table 1. Population parameters used to model the Sable Island and Gulf of St. Lawrence grey seal populations.

			Sable			Gulf		
	Mass (kg)		*M*			*M*		
Age (years)	Male	Female	Male	Female	Birth %	Male	Female	Birth %
0	52	48	0.294	0.294	0	0.446	0.446	0
1	71	63	0.050	0.050	0	0.078	0.078	0
2	92	78	0.050	0.050	0	0.078	0.078	0
3	113	92	0.050	0.050	0	0.078	0.078	0
4	133	106	0.050	0.050	16	0.078	0.078	16
5	151	118	0.050	0.050	71	0.078	0.078	71
6	168	129	0.147	0.050	91	0.236	0.078	85
7	184	138	0.147	0.050	91	0.236	0.078	85
8	197	147	0.147	0.050	91	0.236	0.078	85
9	208	153	0.147	0.050	91	0.236	0.078	85
10	217	159	0.147	0.050	91	0.236	0.078	85
15	246	175	0.147	0.050	91	0.236	0.078	85
20	256	181	0.147	0.050	91	0.236	0.078	85
25	259	183	0.147	0.050	91	0.236	0.078	85
30	261	183	0.147	0.050	91	0.236	0.078	85
35	261	184	0.147	0.050	91	0.236	0.078	85
39	261	184	0.147	0.050	91	0.236	0.078	85

Note: *M*, instantaneous rate of natural mortality; Birth %, percentage of females giving birth.

the analysis is repeated, and the outputs are compiled. We bootstrapped the residuals (i.e., the differences between the data and the model predictions) to estimate confidence limits on the trends of both the Sable and Gulf populations. A sufficient number of replicates had been performed when the plot of estimated variance on population size versus the number of replicates reached an asymptote. Although this occurred after 30 replicates, we used 100 replicates to estimate the confidence limits for the two populations.

Trends in grey seal abundance

Figure 2*a* shows the estimated trend in Gulf pup production when the two-parameter model is fitted to mark–recapture estimates of pup production in 1984, 1985, 1986, 1989, and 1990 (Hammill et al. 1992). The two estimates for each of these mark–recapture studies were derived from independent samples of the ratio of tagged to untagged seals. The model shows that pup production in the Gulf increased slowly until the cull was ended in 1984. Thereafter, the population is estimated to have increased at about 9%/year, with a doubling time of about 8 years. The coefficients of variation (CV) on the estimates of Gulf pup production, from the NLLS fit, were 43 and 32% for initial population and natural mortality, respectively. Uncertainty in the estimated total numbers of Gulf grey seals increased markedly from the last pup production survey in 1990 (Fig. 2*b*). In 1994, the Gulf population was estimated at 68 700 seals, with a 95% confidence range of 42 000 – 100 000 (CV 22%).

Censuses of grey seal pup production on Sable Island were attempted in most years between 1962 and 1990 (Mansfield and Beck 1977; Stobo and Zwanenburg 1990). However, counts prior to 1976 were often based on short visits to the island and, in most cases, underestimated pup production (Zwanenburg and Bowen 1990). Thus, we used only pup censuses conducted between 1976 and 1990 (Stobo and Zwanenburg 1990) to estimate the trend in Sable Island pup production

Table 2. Age-specific, late-term pregnancy rates of grey seals from Mansfield and Beck (1977) used as birth rates in the model.

Age (years)	Number pregnant	Total number	% pregnant
4	3	19	17
5	5	7	41
6+	43	50	86

during the period 1967 to 1994. Pup production estimates prior to 1976 lay consistently below the line as previously reported (Fig. 3*a*). The CVs for the parameter estimates of the Sable Island pup production model were 9 and 7% for starting population and natural mortality, respectively. A preliminary estimate from the 1993 aerial photographic survey (W.T. Stobo, personal communication, see Fig. 3*a*) indicates that pup production on Sable Island continues to increase at about 13%/year. This corresponds to a doubling time of slightly less than 6 years. The Sable population in 1994 was estimated at 85 300 (78 000 – 95 000 95% confidence interval; CV 4%) seals (Fig. 3*b*).

Our analysis suggests that the Gulf and Sable populations have experienced different dynamics over the past three decades, such that there has been a shift in the relative contribution of these populations to the total number of grey seals in eastern Canada. In 1970, we estimate that 73% of the total population was of Gulf origin. By 1982, the two populations were about equal in numbers. However, by 1994, only 45% of the estimated total population (about 154 000 seals) was of Gulf origin.

The dynamics of these two populations over the past several decades are described reasonably well by the two-parameter model. Pup production and hence our estimates of trends in the Gulf population size are much less certain than they are for Sable Island. However, neither population can continue to increase exponentially indefinitely. At some point, density-

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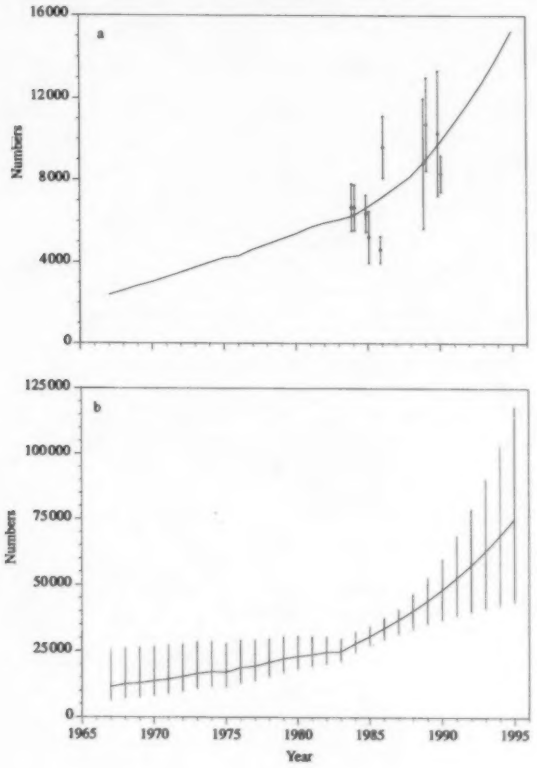
Fig. 2. Estimates and model fit (solid line) of pup production (*a*) and total population size (*b*) of Gulf of St. Lawrence grey seals with 95% confidence limits estimated by bootstrapping.



dependent changes in population parameters will alter current population trajectories. We have not considered density dependence in this study, as the empirical evidence suggests that this factor has not affected the dynamics of these populations.

Seasonal distribution of grey seals in the 4VsW study area

The magnitude of grey seal predation on ESS cod depends, among other things, on the seasonal distribution of grey seals, as the two grey seal populations are known to undertake wide-ranging seasonal movements (Mansfield and Beck 1977; Lavigueur and Hammill 1993; Stobo et al. 1990). The relative contributions of both populations to the total number of grey seals in the study area (Table 3) were derived from a qualitative assessment of the seasonal distribution of tag recoveries from seals marked in the Gulf and on Sable Island (Lavigueur and Hammill 1993; Stobo et al. 1990) and from a general knowledge of the natural history of grey seals where tag return data were clearly deficient. For example, tag recoveries are mainly from coastal locations and from pups and juveniles. Thus, they provide little information on the offshore distribution of grey seals or the distribution of adults, particularly during the breeding season.

Fig.3. Census counts and model fit (solid line) for pup production (*a*) of the Sable Island grey seal population. Only data from 1976–1990 (dots) were used to estimate model parameters. Preliminary aerial survey estimate for 1993 also shown. (*b*) Trend in total population size of Sable Island grey seals with 95% confidence limits estimated by bootstrapping.

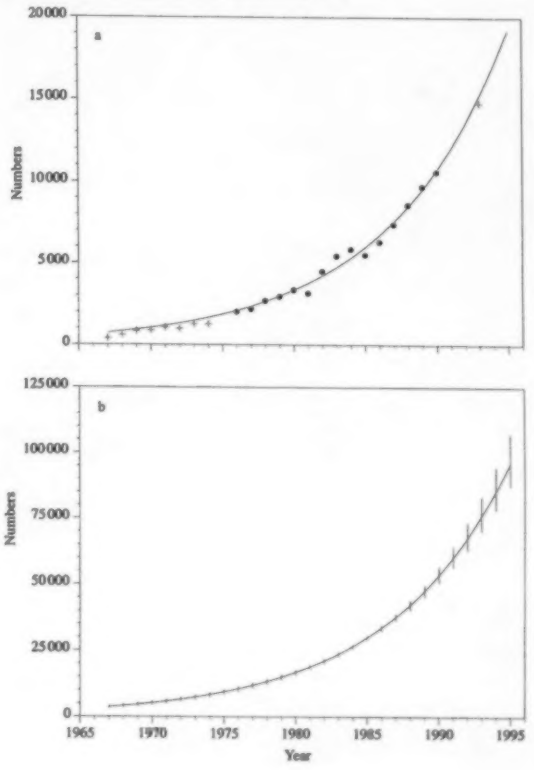


Table 3. Percentage of each population of grey seals in the ESS study area by quarter of the year. First quarter begins January 1.

	Quarter			
Population	1st	2nd	3rd	4th
Sable	90	50	50	80
Gulf	10	20	20	10

Predation models

Seal energy requirements

Estimates of the average daily metabolic rate (ADMR) of free-living grey seals are not available. Castellini et al. (1992) measured the metabolic rates in five free-living, adult Weddell seals (*Leptonychotes weddellii*) while the animals were resting, while they were diving, and during sleep and found that their ADMR was about 1.5–2.0 times the predicted standard metabolic rate for animals of similar size (Klieber 1961).

The only grey seal data come from captive studies. Ronald et al. (1984) found that the standard metabolic rate of adult grey seals was about equal to that predicted by the Klieber

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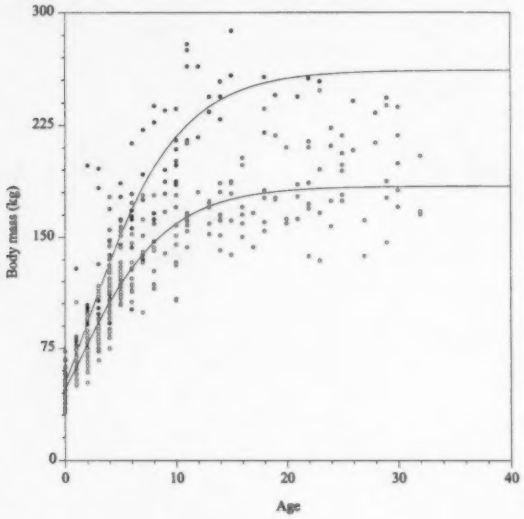
Fig. 4. Gompertz growth models fit to male (solid circles) and female (open circles) grey seal body masses collected from the Gulf population.



equation. Fedak and Hiby (in Anonymous 1984) measured the metabolic requirements of 10 grey seals ranging in age from 1 month to more than 10 years and in body mass from 43 to 270 kg. They estimated that an adult grey seal would expend energy at a rate about twice that predicted by the Klieber equation. Although these data do not include the cost associated with diving, both the Weddell seal results and recent studies on free-ranging grey seals that demonstrate marked reduction in heart rate during diving (Thompson and Fedak 1993; Fedak and Thompson 1993) suggest that the ADMR of adult grey seals is reasonably estimated at two times the Klieber equation.

The mass-specific metabolic requirements of young growing animals are greater than those of adults. Lavigne et al. (1986) concluded that the standard metabolic rate of immature, growing pinnipeds was about two times that predicted by Klieber's equation for adults. Worthy (1987) found that most values of standard metabolic rate in captive, neonatal grey seals lie between two and three times that predicted by Klieber's equation. Ronald et al. (1984) found that the standard metabolic rate of growing 1-year-old and 2-year-old grey seals was 1.8 and 1.7 times the rate predicted by Klieber's equation, respectively. We have assumed that metabolic rate decreases geometrically to the adult rate (i.e., age 6 and older), such that pups through 5-year-old seals require a premium of 200, 150, 125, 112, 106, and 103%, respectively.

We used the following equation to estimate the daily gross energy intake (GEI; in watts per day) of an individual grey seal:

$$(2)\quad GEI(s,a) = GP(a) \times (2 \times 3.4 \times BM(s,a)^{0.75})/(ME - HIF)$$

where s is sex and a is age (0–39 years for males and females), GP is growth premium, BM is body mass (kg), ME is metabolizable energy (i.e., the proportion of GEI available to the animal), HIF is the proportion of GEI lost to heat increment of feeding, and $3.4 \times BM^{0.75}$ is the Klieber equation. The estimated ME in grey seals fed herring averaged 83 ± 4.8% of gross energy intake (Ronald et al. 1984). There are no estimates of the HIF in grey seals. Instead, we used recent data from captive harbour seals showing that an average of 8.3% of GEI is lost to HIF (Markussen et al. 1994). Although it could be argued that HIF is accounted for in the Klieber multipler of 2, we identified HIF as a term in the equation so that we could assess the sensitivity of our estimates to variation in this parameter.

Table 4. Parameter estimates and coefficients of variation (CV) of the Gompertz fits to body masses of male and female grey seals.

	p_1	CV %	p_2	CV %	p_3	CV %
Male	51.958	5.5	1.615	3.2	0.217	6.7
Female	47.796	4.4	1.346	3.1	0.223	5.5

We made a number of simplifying assumptions in calculating the energy requirements of the two grey seal populations. In particular, we did not explicitly account for seasonal variation in energy requirements associated with the cost of reproduction and moult. This approach was taken for several reasons. First, the construction of detailed individual energy budgets requires the estimation of a large number of parameters and for a number of these parameters little information exists. Therefore, the inclusion of such detail in many cases results in a loss of precision in the model. Also, although reproduction is costly, much of this expense is cancelled by the reduced energy requirements during the moult (Ryg and Øritsland 1991). Finally, when expressed in terms of energy requirements of the population, the error in not explicitly modelling the cost of reproduction is about 5% (Olesiuk 1993).

The age-specific body masses of male and female grey seals were determined from samples collected in the Gulf of St. Lawrence between 1988 and 1992 (M. Hammill, Maurice Lamontagne Inst., Mont Joli, Que., unpublished data) (Fig. 4). These data were fit using an NLLS and the Gompertz equation in the form

$$(3)\quad BM = p_1 \times \exp(p_2(1 - \exp(-p_3 \times a)))$$

where BM and a are as defined previously and p_1–p_3 are parameters to be estimated. These growth curves (Table 4) were used for both the Gulf and Sable Island populations.

Prey size and composition of grey seal diets

The percent wet mass of ESS cod and other prey in the diet of grey seals was estimated from otoliths and other hard parts recovered in 198 grey seal stomachs from inshore sites along the eastern shore of Nova Scotia and 393 faecal samples collected on Sable Island during 1988–1993 (Bowen et al. 1993; Bowen and Harrison 1994; W.D. Bowen, unpublished data; Table 5). Atlantic cod constituted 1.6–44.1% by mass of the diet in the above studies. Although these estimates varied considerably, there was little evidence of an interannual trend in the percentage of cod in the diet despite marked variation in the abundance of ESS cod over the same time period. The average energy density of the 13 diet samples varied from 5.0 to 8.8 kJ/g wet mass.

There are no quantitative data on grey seal diets on the Scotian Shelf prior to 1988. Although estimates of percent wet mass are not available, we do have frequency of occurrence

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Table 5. Estimates of percent wet mass and energy density of grey seal diets from stomachs and scats collected on the Scotian Shelf from inshore (in) and offshore (off) locations in 1988–1993.

	1988–1990				1992	1991			1992				1993		
	Summer		Winter		(summer)	July	Sept.	Nov.	Feb.	Mar.	May	Aug.	(Jan.)	Energy	
	(In)	(Off)	(In)	(Off)	(in)	(off)	(off)	(off)	(off)	(off)	(off)	(off)	(off)	kJ/g	Source
Cod	17.6	6.1	13.9	20.1	33.5	19.2	44.1	12.7	3.5	4.1	1.6	17.2	3.7	4.2	1
Haddock					2.6									5.4	2
Pollock	7.0	6.8												5.0	3
Redfish						1.2		0.4	2.7	2.4	0.7	1.5	0.4	4.2	1
Silver hake	3.2	25.8	0.7	0.6	2.5	5.0	3.1	1.5	0.4	0.2	0.1	5.6		4.2	1
Flatfish					14.6	18.5	2.9	7.8	10.7	15.7	19.4	7.5	7.9	4.2	1
Sand lance		32.7		75.1		56.0	49.7	75.1	78.7	76.1	77.8	67.2	85.3	6.7	4
Herring	65.8		9.0		33.2									10.9	2
Mackerel	0.4		47.4		9.9									10.0	5
Squid	1.4	26.6	12.9	4.2										4.2	5
Total	95.4	98.0	83.9	100	96.3	99.7	99.8	97.5	96.0	98.5	99.6	99.0	97.3		
Average energy	8.8	5.0	8.4	6.3	7.1	5.4	5.4	6.3	6.3	6.3	6.3	5.9	6.3		
Sample type	Stom	Stom	Stom	Stom	Stom	Scat	Scat	Scat	Scat	Scat	Scat	Scat	Scat		
n	90	26	16	11	55	50	52	49	41	54	43	59	45		

Note: Stom, stomach sample. Source: 1, W.D. Bowen and S.J. Iverson, unpublished data; 2, Murie and Lavigne (1992); 3, Jensen (1979); 4, Birkhead and Nettleship (1987); 5, Bowen et al. (1993).

Table 6. Estimates of percent relative frequency of occurrence of cod in grey seal diets between 1969 and 1987.

Years	Location	% relative frequency of occurrence of cod	Source
1969–1971	Atlantic Canada	16.9	Mansfield and Beck 1977
1963–1987	Nova Scotia	3.6	Benoit and Bowen 1990
1983–1984	Sable Island	21.3	Benoit and Bowen 1990

data from eastern Canada for earlier periods (Table 6). These data indicate that cod was frequently consumed by grey seals from 1970 to 1993. Frequency of occurrence data underestimate the importance of cod in the diet of grey seals compared to relative percent wet mass (Benoit and Bowen 1990; Bowen et al. 1993). Thus, these limited data suggest that cod may have represented a larger fraction of the grey seal diet in the past, when ESS cod were more abundant. We used this observation as a rationale for the development of the proportional ration model.

Our estimates of diet composition are based mainly on the identification of otoliths and squid beaks. Undigested prey are occasionally found in stomachs, but they constitute <1% of the reconstructed mass of food eaten. Prey without hard parts and those with hard parts that are not resistant to digestion will be underestimated in the diet by this method. This will tend to overestimate the importance of species that have robust otoliths, such as cod and other groundfish. Although we have no way of knowing how serious this bias may be, we have adjusted the average energy of the diets given in Table 5 on the assumption that 20% of the diet by mass may not have been seen. We assumed that the average energy density of this unseen prey was 4.2 kJ/g wet mass, a value that would be appropriate for squid and small gadoids and flatfishes whose otoliths are unlikely to survive digestion (Table 5).

The relationships between otolith length and body length of ESS cod and other prey were used to estimate the size of prey consumed by grey seals (Bowen et al. 1993; Bowen and Harrison 1994). We used three length-classes of cod in our analysis (<30, 31–45, and >45 cm). The average mass and length at age of Atlantic cod were determined from groundfish bottom trawl surveys conducted over the period 1970–1993 (Mohn and MacEachern 1994). The cut points of the three length-classes (30 and 45 cm, Table 7) fell midway in the length distribution of the 2 year olds and the 4 year olds, respectively, as determined from the July groundfish survey. The proportion by number of cod at age, within a size-class, was used to estimate the average mass for each of the three size categories: 0.12, 0.50, and 1.80 kg, respectively (Table 7). These masses were multiplied by the percentages of cod for each size category (79, 16, and 5%) from the seal diet studies (Bowen et al. 1993; Bowen and Harrison 1994) to give the percentage by mass (36, 30, and 34%) of cod in the diet. In the model, we assume that the percentage in each of these three length-classes of ESS cod eaten by grey seals is constant over time, although the percentages would change owing to varying year-class strengths and variation in growth. To convert the removals by seals from the three length categories into age categories the process was essentially reversed. The numbers eaten in each size category were partitioned into age-classes according to the proportions in the first three columns of Table 7.

Models of seal food consumption on the eastern Scotian Shelf

The functional response of grey seals to changes in prey abundance is unknown. Therefore, we use two models to describe ESS cod consumption by grey seals. We call these the constant ration and the proportional ration models. The constant ration model assumes that there is sufficient prey such that ESS cod

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Table 7. Proportion of numbers in each length category and mean length and mass at age of ESS cod from July bottom-trawl roundfish surveys.

Age (years)	Length category 0–30 cm[a]	31–45 cm[a]	>45 cm[a]	Mass (kg)	Length (cm)
1	0.75	0.00	0.00	0.08	20.3
2	0.25	0.39	0.00	0.25	29.1
3	0.00	0.47	0.00	0.56	38.2
4	0.00	0.14	0.27	0.98	46.2
5	0.00	0.00	0.33	1.51	53.5
6	0.00	0.00	0.20	2.10	59.8
7	0.00	0.00	0.12	2.78	65.6
8	0.00	0.00	0.07	3.75	72.2

[a]Derived from smoothed average survivorship of cod from the VPA.

represent a constant fraction of the grey seal diet irrespective of the abundance of ESS cod. Although this might seem to be an unrealistic model, cod comprise only a small fraction of the diet and thus grey seal predation may not be closely coupled to the abundance of cod. In fact, our data on the percentage of cod in the diet of grey seals between 1989 and early 1993 suggest this relationship (Table 5). In the second model, we assume that grey seal predation is proportional to the biomass of ESS cod (similar to a Lotka–Volterra relationship), that is

$$(4) \quad E_s = q_s \times B_s \times N'$$

where E_s is the mass (tonnes) of cod eaten in size-class $s = 1$–3, B_s is the estimated 4VsW cod biomass in the three length-classes, q_s is a scaling coefficient, and N' is the number of seals scaled by age and sex for their energy requirements. By finding the average cod biomass (3875, 21 725, 65 650 t for cod length-classes 1–3, respectively), the amount eaten by seals (3367, 2806, 3189 t), and the average number of seals (38 730) in 4VsW over the 4-year period (1989–1992) covered by the diet study, the average q_s values are determined (2.25, 0.33, 0.12; $\times 10^{-5}$). These q_s values are then held constant for the entire simulation period and the amount of cod consumed in each quarter is calculated and accumulated into annual totals. This model essentially assumes that the availability of all other food items is irrelevant and that when the cod biomass doubles, its abundance relative to other prey also doubles. This assumption is required because the abundance of most other prey is not known.

Grey seal predation on each length-class of cod and the total biomass of prey consumed were estimated quarterly. The fractions of the Gulf and Sable grey seal populations in the study area each quarter were taken from Table 3. The percentages of ESS cod in the diet were then calculated depending upon the ration model chosen. The values for each of the length-classes of ESS cod in the diet were converted from tonnes into numbers of fish as described above using the values in Table 7. Daily GEI of the grey seal population was calculated for each sex by multiplying numbers of seals at age corrected for survivorship by the appropriate GEI(s, a) and then summing over ages. The daily GEI was converted from mass of food eaten into a quarterly energy requirement in tonnes by dividing the requirements by the average energy density of the prey (Table 5) and summing over the number of days per quarter. We use population numbers in 1994 as an example of the age- and sex-specific daily energy requirements, and daily and annual food consumption of grey seals (Table 8). Because of the effect of the growth premium on energy requirements and, more importantly, because of the greater abundance of younger seals, the first five age-classes (ages 0–4) represent over half the population food consumption.

The total annual food intake by grey seals in the study area increased from an estimated 10 000 t (8100 to 12 600 95% CI) in 1970 (rounded to the nearest 1000 t) to 121 000 t (112 000 to 133 000 95% CI) in 1994. In the constant ration model, the assumption that 20% of the diet was unseen decreased the percentage of cod in the diet by 25% and the total energy intake by about 8%, resulting in an increase in quarterly food intake of about 9%. For example, the estimated quarterly food intake of a 4-year-old male would increase to 0.59 t after the correction for unseen prey from 0.54 t before the correction. On average, approximately 15% of the adjusted grey seal diet was cod.

The estimated biomass of ESS cod consumed by grey seals in the constant ration model increased steadily from an average of about 1250 to 15 400 t over the period 1970–1994 (Fig. 5). In the proportional ration model, the estimated consumption of ESS cod by grey seals was low in 1970, increased to a peak during the period of high cod abundance during the early 1980s, and then gradually declined to about 6300 t in 1994 (Fig. 5). The directed fishery for cod was closed in September 1993 and the 1994 landings were less than 400 t.

The same overall patterns are observed when both landings and grey seal predation on cod are expressed in terms of numbers of fish. Under the constant ration model, grey seals are estimated to have consumed about 5 million ESS cod in 1970, increasing to about 60 million cod by 1994, comprising mostly fish <30 cm in length. By contrast, under the proportional ration model, grey seals consumed about 25 million ESS cod in 1970, about 53 million during the early 1980s, and about 23 million cod in 1994.

Sensitivity and uncertainty of the predation model

Our estimates of grey seal predation mortality on cod depend on the precision of the data used in the model (i.e., sampling error) and the validity of our assumptions where data are unavailable (i.e., model error). We used two approaches to assess the sensitivity of model estimates to uncertainty in input parameters: bootstrapping and changing the magnitude of input parameters. For the food consumption models, bootstrap experiments were performed that explicitly resampled the underlying data. To assess the contribution of components of the bioenergetic equation (eq. 2) to uncertainty in food consumption, we bootstrapped data on the percentage of cod in the diet ($n = 13$, Table 5), the average energy density of the diet ($n = 13$, Table 5), the heat increment of feeding ($n = 19$, Markussen et al. 1994), and metabolizable energy ($n = 9$, Ronald et al. 1984). Variation in grey seal mass at age from the growth model (Fig. 5) was estimated by conditioned bootstrapping (100 replicates) on the residuals from the Gompertz model. The variance in the total food consumption was most affected by the average energy of the diet, about a 4% CV (Table 9). When all the sources were resampled the CV of the total food consumption was 5.1%. The CV for the amount of cod eaten was about 21% when the percentage of cod in the diet was bootstrapped and less than 5% for the other parameters. When

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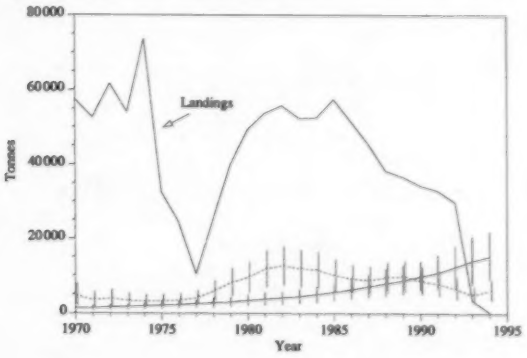
Table 8. Age- and sex-specific numbers, daily energy intake, daily food intake, and annual food intake of the grey seals in the study area in 1994.

	Number in 1994		Daily energy per individual (W)		Daily food per individual (g)		Annual population consumption (t)	
Age (years)	Male	Female	Male	Female	Male	Female	Male	Female
0	5 658	5 658	353	331	5028	4723	10 391	9 760
1	4 365	4 365	335	304	4777	4335	7 616	6 911
2	3 701	3 701	338	298	4816	4245	6 509	5 738
3	3 136	3 136	354	305	5052	4348	5 787	4 981
4	2 657	2 657	378	319	5384	4543	5 225	4 409
5	2 250	2 250	405	336	5777	4795	4 748	3 940
6	1 812	1 907	426	349	6075	4974	4 021	3 463
7	1 391	1 622	454	368	6478	5244	3 292	3 106
8	1 068	1 379	478	384	6822	5471	2 660	2 756
9	818	1 170	499	397	7112	5659	2 124	2 419
10	626	994	516	408	7354	5815	1 683	2 111
15	163	433	565	439	8059	6255	481	990
20	43	191	583	449	8311	6406	130	447
25	11	85	589	453	8398	6456	35	200
30	3	39	591	454	8428	6473	9	92
35	1	17	592	454	8438	6478	2	40
39		9	592	454	8441	6480		21
Total	29 486	34 270	534[a]	420[a]	7622[a]	5984[a]	59 837	61 943

[a]Column average.

Fig. 5. Commercial landings (t) and biomass (t) of ESS cod eaten by grey seals with 95% error bars for the constant (solid line) and proportional (broken line) ration models.



all five sources were resampled together the CV on the amount of cod eaten per seal was about 22%.

The error bars in Fig. 5 combine the uncertainty in the estimation of both population size and the percentage of cod in the diet, under the constant ration and proportional ration models. When both sources of uncertainty were bootstrapped, the CV of food consumption by grey seals was 23.1%. When only the percentage of cod in the diet was bootstrapped, the CV was 22.3%, illustrating the importance of this source of uncertainty for estimates of cod consumption. The contribution from the variance in the population alone was 5.4%.

The sensitivity of the model to various parameters or state variables was examined by independently changing parameters used in the predation model by 10% (Table 10). The measure we used is known as the relative sensitivity (R), which is

Table 9. Bootstrap coefficients of variation (%) for components of the grey seal consumption model.

Parameter sampled	Total consumption	Cod consumption
% cod eaten	1.0	20.9
Average energy of diet	3.9	3.9
HIF	1.0	1.0
ME	2.1	2.1
Gompertz parameters	1.5	1.5
All of the above	5.1	22.2

the proportional change of an output (O) divided by the proportional change of a parameter or intermediary variable (X) and expressed as a percentage:

$$(5) \quad R = 100 \times (\Delta O/O)/(\Delta X/X)$$

First, a base run was made with unperturbed parameters and the outputs, cod and total consumption in 1994, were estimated. Then, one of the parameters, for example the HIF, was increased by 10%. The model was run again and the amount of cod and total prey consumed were then compared with the base run and the relative difference was computed. In this example the relative sensitivity was 26% for either cod or total consumption.

The estimated cod consumption by grey seals was quite sensitive to the size of the Sable Island population, the fraction of GEI available to the animal, the percentage of cod in the diet, the energy density of the diet, the Klieber multiplier, and the body mass of seals. Given our assumptions about the quarterly distribution, within the study area, the model results were relatively insensitive to a change in the size of the Gulf population. However, the quarterly distribution of the two populations was quite uncertain and this uncertainty translated directly into uncertainty in the effective population size and

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Table 10. Relative sensitivity (%) of cod and total annual food consumption to a 10% increase in input parameters.

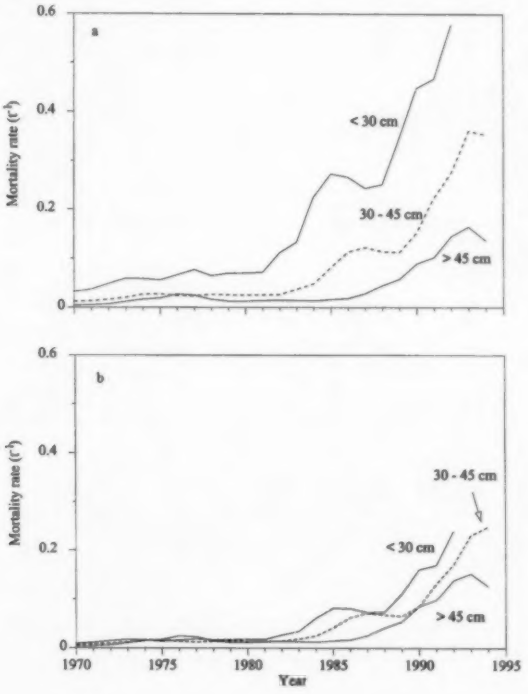
Parameter	Base value	Cod consumption	Total consumption
Sable numbers	Fig. 3	83	83
Gulf numbers	Fig. 2	17	17
% Sable in ESS, 2nd quarter	50	15	15
% Gulf in ESS, 2nd quarter	20	6	6
% unseen prey	20	–9	7
Growth premium	See text	63	63
ME	0.83	–112	–112
HIF	0.083	26	26
% cod in diet	15.2	100	0
Average energy density of diet	1.45	–83	–83
Klieber multiplier	2	100	100
Seal mass at age	From Table 1	74	74

Table 11. Summary of yield per recruit and potential yield estimates of the 1990 year-class of ESS cod for two types of natural mortality (M_C and M_{AS}) and with or without predation by seals.

	M_C		M_{AS}	
	No seals	Seals	No seals	Seals
Recruits in 1990 year-class (millions)	25	72	183	303
F0.1*	0.174	0.480	0.174	0.481
Yield per recruit at F0.1 (g)	400	98	54	25
Potential yield for 1990 year-class (t)	10.08	7.03	9.96	7.70

*F0.1 is a target fishing motality (Gulland and Boerema 1973).

Fig. 6. Average annual predation mortality rates by grey seals on the three length-classes of ESS cod under two assumptions about the instantaneous rate of residual natural mortality (M): (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific). There is no estimate of the F on the smallest size-class for 1993 and 1994 because by convention 3 years is the youngest age fit in the VPA model for this cod stock.



thus the consumption of cod and other prey. Both cod and total food consumption were also insensitive to a change in the correction factor for prey that may not have been detected by the methods used to determine diet composition. Although we changed each parameter independently to isolate their effects, in reality they will often covary; for example, the percentage of cod may covary with the average energy density of the diet.

Effects of seal predation on cod

Predation by grey seals can be viewed as an analog to the catch from a fishery. In this section we compare the mortality of cod from predation by grey seals with that generated by the commercial fishery. The effect of the commercial fishery on the ESS cod population was assessed annually (Fanning et al. 1995) using virtual population analysis (VPA). The fishery data covered the period 1970–1994 and cod 1–15 years of age. Landings at age and survey numbers at age were fit to a population model using an NLLS procedure (Fanning et al. 1995). The landings at age were converted into cod numbers at age subject to assumptions about natural mortality (M). The rate of natural mortality of cod, which is only poorly known, is a key parameter of the cod VPA model. By convention, it is assumed that the M from all sources, including grey seals, of ESS cod is 0.2 for all ages. When estimating the rate of seal predation mortality, assumptions about the magnitude and age-specific pattern of M become quite important.

There have been few attempts to measure age-specific M in cod. Some authors (Beverton and Holt 1959; Pauly 1980) inferred M from other biological characteristics such as life-span and growth rate. Sparholt (1990) and the ICES Working Group on Multispecies VPA investigated this issue in North Sea groundfish. This working group (Anonymous 1993) reported M values of 0.8, 0.35, and 0.25 for ages 1–3 and 0.2 for age 4 and older for North Sea cod on the basis of fish diets and a multispecies VPA. Because ESS cod grow more slowly than North Sea cod (for example, an age-3 North Sea cod at 2.6 kg weighs more than a 9-year-old cod from 4VsW) and in the absence of data on ESS cod, we assumed that M of juvenile ESS cod is higher and persists at high levels for 1 year longer than reported for North Sea cod. We used the following vector of M values: 1.0, 0.8, 0.6, and 0.4 for ages 1 to 4, respectively, and 0.2 for ages 5–15. To assess the impact of these two assumptions about other sources of M on estimates of seal predation mortality, we ran all analyses twice, once with $M = 0.2$ across all ages and again using the age-specific vector. Hereafter, we will denote the constant natural morality as M_C and the age-specific version as M_{AS}. Figures 6–12 compare results

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Fig. 7. Instantaneous total (Z), fishing (F), and grey seal predation (M_S, under the constant ration model) mortality rates on ESS cod averaged over ages 2–7 with explicit grey seal predation in the VPA. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).

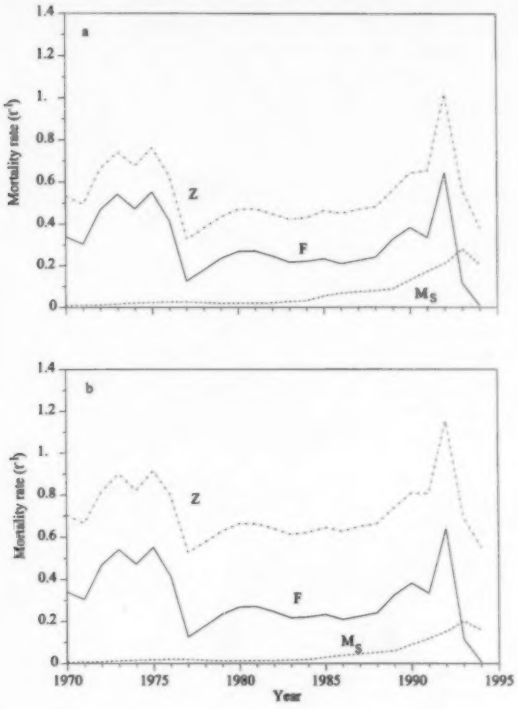
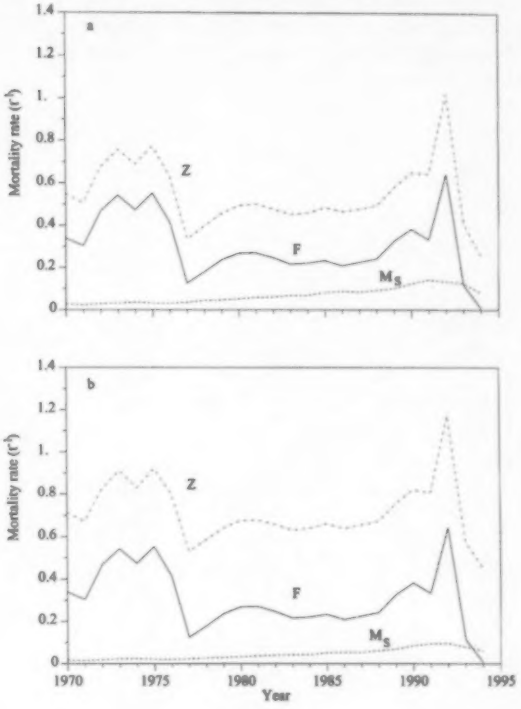


Fig. 8. Instantaneous total (Z), fishing (F), and grey seal predation (M_S, under the proportional ration model) mortality rates on ESS cod averaged over ages 2–7 with explicit grey seal predation in the VPA. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).



under the two assumptions of cod natural mortality. Although we cannot be sure that they represent extremes, the comparison represents a wide range of possibilities.

Seal predation mortality

Grey seal predation mortality (M_S) of ESS cod was estimated by first running a standard VPA using the commercial landings and research trawl survey data as inputs and then performing the NLLS fit as described in Fanning et al. (1995). This resulted in estimates of age-specific population numbers and fishing mortalities of cod. The consumption of ESS cod by grey seals was then added to the commercial landings and the VPA was repeated. The VPA was then tuned again to account for the removals by grey seals. This combined cod mortality was then partitioned into deaths from the commercial fishery and those from grey seal predation. For this and the remainder of the analyses in this paper, we used both the constant and proportional ration models of grey seal consumption.

If seal predation mortality were additive, both cod recruitment at age 1 and population size would increase because the sum of M_S and M_R (i.e., residual M from sources other than seals) would be larger than M alone. However, at present we do not know how much compensation might occur among the components of M. Therefore, to allow more direct comparisons both with the standard VPA results and to reflect our uncertainty about the interaction of components of natural mortality, M was reduced so that the average recruitment of cod from 1970–1974 was the same (i.e., within 1%) with and without seal predation under the constant ration model. This meant reducing M to 85% of M_C and reducing M to 97% of M_{AS} before adding in the seal predation component of natural mortality, M_S. We did not follow this procedure in the estimation of yield per recruit to emphasize the potential contribution of additive seal predation mortality in each decade.

Under the assumption that $M = M_C$, the smallest length-class of cod consistently had the highest seal predation mortality rate, increasing rapidly in the early 1980s to about 0.58 in 1992 (Fig. 6*a*). The rate of seal predation mortality on larger cod was generally <0.05 until the late 1980s when mortality rose quickly as the cod stock declined. When M_{AS} was assumed, the estimated mortality rate caused by grey seals dropped considerably on the smaller size-classes of cod and did not begin to increase until the mid to late 1980s (Fig. 6*b*). This result was expected, because predation by seals was now being compared with a higher residual natural mortality, which implies a larger cod population in the VPA. Nevertheless, even in this case, total mortality owing to seals was >0.2 by the late 1980s. Given that grey seals prey mainly on small cod, seal predation mortality on larger cod was largely unaffected by our assumptions about M (Fig. 6).

The mortality attributable to seal predation may be put into context by comparing it with both fishing and total mortality (Figs. 7 and 8). Mortalities owing to seals and owing to the fleet were averaged over ages 2–7. The reduction in total mortality

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Fig. 9. VPA estimates of age-1 recruitment in ESS cod for 1970–1992 with (solid line) and without (dotted line) explicit seal predation using the constant ration model. Projected mean recruitment and 95% confidence limits for the period 1993–1995 are indicated by the solid-line series (see text for details). The dashed-line series are the model predictions of recruitment after grey seal predation had occurred. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).

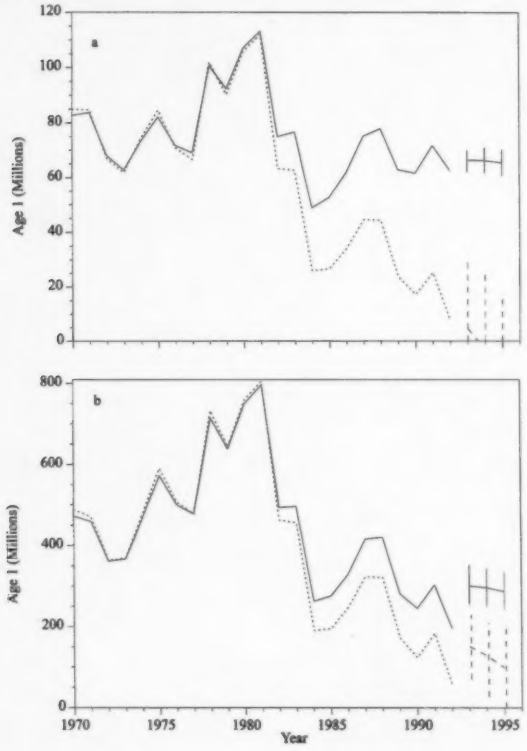
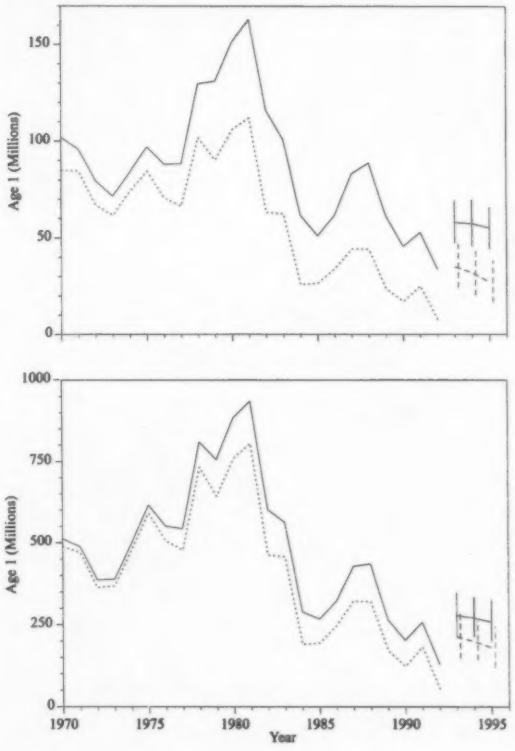


Fig. 10. VPA estimates of age-1 recruitment in ESS cod for 1970–1992 with (solid line) and without (dotted line) explicit seal predation using the proportional ration model. Projected mean recruitment and 95% confidence limits for 1993–1995 are indicated by the solid-line series (see text for details). The dashed-line series are the model predictions of recruitment after grey seal predation had occurred. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).



of ESS cod seen in the late 1970s was due to the nearly total exclusion of foreign fishing effort in 4Vs and 4W with the extension of Canadian jurisdiction to 200 miles. In the constant ration model (Figs. 7*a* and 7*b*), under either assumption about residual *M*, the contribution by grey seals to total mortality was small until the late 1980s but became more pronounced in the 1990s as the number of grey seals continued to increase while the cod population rapidly decreased in size. Interestingly, seal predation mortality on the 2- to 7-year-old cod levelled off in 1994, despite a continued increase in the number of grey seals. This was probably caused by the presence of the 1990 year-class, which was large compared with adjacent year-classes. Under the proportional ration model (Figs. 8*a* and 8*b*), grey seal predation mortality increases slowly throughout most of the period, but is still low compared with fishing mortality.

Recruitment

We examined the potential impact of grey seal predation on estimates of recruitment (i.e., numbers at age 1) in the population by running the VPA without and with explicit seal predation. The dotted line in Figs. 9 and 10 from 1970–1992 represents the estimated recruitment from the VPA when grey seal predation is not explicitly included in the model assumptions about *M*. In this standard version of the VPA (e.g., Fanning et al. 1995), recruitment estimates of ESS cod declined rapidly during the 1980s and early 1990s (Figs. 9 and 10). However, when seal predation was explicitly added to this model (i.e., accounting in the VPA for the age-1 cod estimated to have been eaten by grey seals) recruitment at age 1 appeared not to have been so severely reduced during this recent period, although the effect was less in the proportional ration model (Fig. 10*a*). Under the assumption of M_{AS}, in both the constant (Fig. 9*b*) and the proportional (Fig. 10*b*) models, the effect of grey seal predation on apparent recruitment was reduced because the predation took place on a population in which the number of recruits was about 6 times greater owing to the higher mortality at young ages.

Stock-recruitment relationship

The addition of explicit seal mortality to the VPA also affected the stock-recruitment pattern of ESS cod (Fig. 11). In interpreting Fig. 11, it is important to remember that the underlying assumption in the model is that seal predation is additive and

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Fig. 11. Stock-recruitment relationships with (solid dots) and without (numerals) grey seal predation added to the ESS cod VPA. For each year, the number locates the parent stock and corresponding recruitment in the absence of explicit seal predation. This number is joined by a line to a dot, which is the same year's position after including predation by grey seals. The underlying assumption in the model is that seal predation is additive. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).

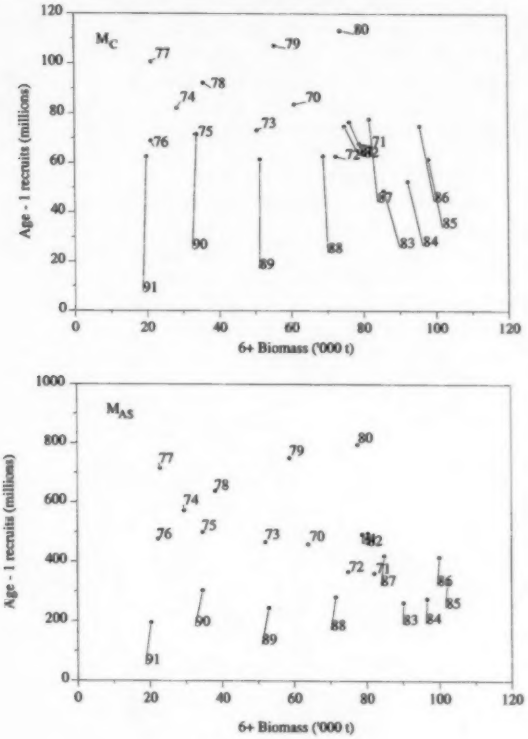
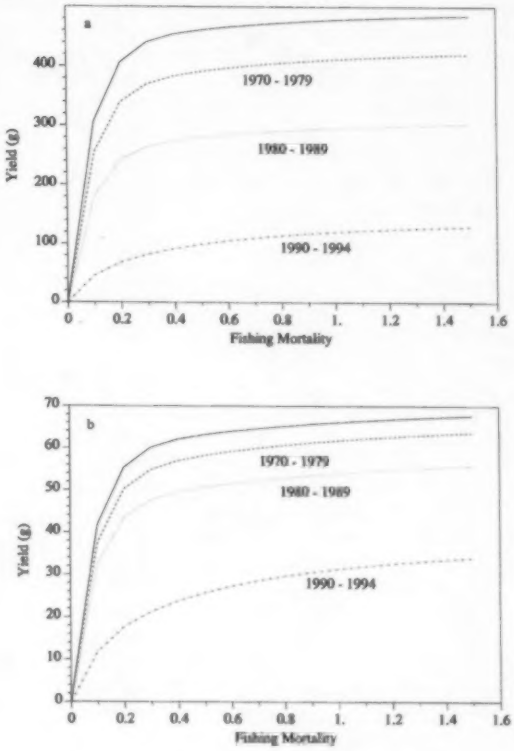


Fig. 12. Yield per recruit of ESS cod without grey seal predation (solid line) and incorporating grey seal predation mortality for 1970–1979, 1980–1989, and 1990–1994. (*a*) $M = M_C$ (constant) and (*b*) $M = M_{AS}$ (age specific).



independent of cod abundance. If there were strong compensation in the components of *M*, then the effect of predation on the estimated stock and recruitment would be less (i.e., the length of the lines shown in these figures would be shorter). Thus, the end points of each line are the extremes of the degree of compensation for seal mortality in the predator field.

The lines are too short to be visible for the early years, but under either assumption about *M*, the lines increase in length over time. Without explicit seal predation, recruitment fell significantly through the 1980s despite a high biomass of spawners and continued to fall at moderate spawning stock size in the late 1980s. Adjusting for the effects of grey seal predation substantially increased the initial size of the 1983–1990 year-classes. When $M = M_C$ was assumed, recruitment seems to have remained reasonably strong during the 1980s, but only a small fraction of these young fish survived to commercial size. This effect was less pronounced if M_{AS} was assumed. However, in neither case did seal predation account for the decrease in year-class size during much of the 1980s compared with that observed during the 1970s, suggesting an increase in other sources of natural mortality or reduced spawning success.

Yield per recruit

The potential impact of grey seal predation on the yield of ESS cod was examined through a yield per recruit analysis (Thompson and Bell 1934). The yield per recruit model balances cod growth with removals and deaths at age. Yield per recruit of cod may have been reduced by >50% as a result of seal predation in the most recent period (Figs. 12*a* and 12*b*), provided that seal predation mortality was additive. Note that the magnitude of the yield varied by a factor of about 7 under the two assumptions about *M*.

These yield per recruit comparisons must be interpreted with caution because the relative importance of different sources of natural mortality on ESS cod cannot currently be quantified. The grey seal component has been explicitly estimated, but it is not known how other sources of natural mortality might respond to a change in the number of seals. However, it is likely that there would be some level of compensation in that if one predator is reduced, the mortality caused by other predators may respond to the increase in food availability. Again, the dotted curves in Fig. 12 are based on the assumption that grey seal M_S is additive. If, on the other hand, there was complete compensation for the increase in seal predation by a reduction in other sources of *M*, then grey seal predation would have had no effect on yield per recruit. Clearly, neither of these extremes is likely and thus the effect

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of grey seals on yield per recruit of cod will probably fall between the dotted curves and the solid curve in Figs. 12*a* and 12*b*.

The implications of the yield per recruit calculations are best evaluated in the context of the effect that explicit seal predation mortality has on levels of recruitment. This is because the reduction in the yield per recruit is partially compensated for by an increase in the level of recruitment implied by seal predation (see Figs. 9 and 10) and the potential yield from a year-class is the product of the yield per recruit and the size of a year-class. For example, under the assumption of $M = M_C$, the estimate of the 1990 year-class more than doubles when seal predation is included in the model, whereas the yield per recruit decreases to approximately 25% (Table 1). The net effect is about a 30% loss in potential yield from this year-class. Implicit in this calculation is that fishing, seal predation, and residual natural mortality patterns remain constant over the period that this year-class would have been in the fishery (i.e., until about the year 2000). Similar results are obtained under the assumption of M_{AS}.

Potential effect of grey seals on future recruitment

To examine the impact of grey seal predation on future cod recruitment, we compared estimates of recruitment from VPA models that explicitly included seal predation, such that $M = M_R + M_S$, with models in which seal predation is an unspecified part of M. For example, in the standard VPA (Fanning et al. 1995), $M = 0.2$ includes seal and all other sources of natural mortality (e.g., dotted line in Fig. 9*a*). To determine the projected recruitment in 1993–1995 that would have occurred if seal predation had not, we bootstrapped the age-1 recruitment estimates from the VPA in which seal predation is modelled explicitly for the period 1982–1992. Confidence limits on the projected recruitment were based on 500 bootstrap replicates. Four projected recruitment series were generated using the two assumptions about M_R (i.e., M_C and M_{AS}), which affect the size of the estimated cod population, and the two ration models. An exponential weighting function with a time constant of 0.2 was applied to the recruitment estimates so that drawing the estimate from 1992 was about 7 times more probable than drawing the estimate from 1982. This form of projection was chosen because of the lack of a clear stock-recruit relationship and the reduced recruitment levels experienced by this stock in recent years. In each panel of Figs. 9 and 10, the upper series of projected recruitments for 1993–1995 are model estimates of the number of age-1 cod that are predicted to recruit to the population when the number of cod eaten by grey seals is accounted for explicitly in the VPA.

Grey seal population numbers and cod consumption were projected to 1995 using the population parameters in Table 1. A predictive relationship between the removals by seals each year and the difference between the two recruitment series (i.e., with and without explicit seals) was derived to estimate future differences between the two recruitment series given an estimate of grey seal consumption in each year. This was done by fitting a linear regression between these two variables for the period 1982–1992 for each of the four recruitment scenarios above. The model fit the data well; all regressions were significant at $p < 0.001$. The predicted differences in recruitment were then subtracted from our projected recruitment to predict the "realized" recruitments for 1993–1995 after explicit grey seal predation had occurred. These realized recruitments (the dotted lines in Figs. 9 and 10) may be compared with the trend in recruitment from the standard VPAs (i.e., where seal predation is implicit). Because the lower series of projections has uncertainty owing to both the recruitment model and the predation model, its error bars are larger than those of the upper series, which include only the cod recruitment uncertainty.

The magnitude of the difference between the projected level of recruitment and that realized after seal predation depends both on our assumptions about the magnitude of residual mortality and on the form of the ration model (Figs. 9 and 10). In the constant ration model, future cod recruitment is significantly reduced by grey seal predation under either assumption about residual M (Figs. 9*a* and 9*b*). In fact, the most extreme prediction is that recruitment would fall to near zero (Fig. 9*a*) following the trend that is seen in the standard (i.e., implicit seal predation) VPA. The effect of grey seal predation is less in the proportional ration model. This is not surprising because in the proportional model grey seals eat fewer cod as cod become less abundant, but even here the model predicts significantly fewer recruits in the face of seal predation if $M = M_C$ (Fig. 10*a*). Only in the case of $M = M_{AS}$ did the model predict no significant reduction in cod recruitment by grey seal predation (Fig. 10*b*).

Discussion

Our analyses, indeed ecological models in general, may be viewed as a serial process that builds on each previous step by adding some new information to form the next; the number of seal pups plus vital parameters is used to estimate the number of grey seals, the number of seals plus their ration leads to estimates of cod consumption, a comparison of this consumption to the size of the cod stock leads to estimates of predation mortality, and finally the predation mortality plus a cod recruitment model leads to an estimate of impact. In the initial stages of this process (for example, the grey seal population models) there are often sufficient data to use bootstrapping to examine the uncertainty of the results. We realize that there are other sources of error for which data do not exist and thus our error bars must be viewed as minima. In the later steps of the analysis, uncertainty is dominated by model assumptions and we have used alternative models to explore the consequences of this uncertainty.

The grey seal population in eastern Canada has increased substantially over the past several decades and there can be little doubt that the number of grey seals inhabiting the study area has also increased. There is greater uncertainty in this latter statement because grey seals are capable of large-scale seasonal movements and our knowledge of these movements is scanty at present. The simple bioenergetic model used to determine grey seal food requirements produced estimates of the daily energy requirements of an average grey seal (7560 kcal/day) that are 29% higher than those in grey seals in the United Kingdom (5860 kcal/day) that were derived from a more complex energetic model (Fedak and Hiby in Anonymous 1984). Much of this difference is accounted for by the larger size of Canadian grey seals (Murie and Lavigne 1992) and by differences in the age structure of the modelled British and Canadian populations. Although additional data on the loss of ingested energy in urine, faeces, and heat would result in modest revision of our estimates, significant improvement

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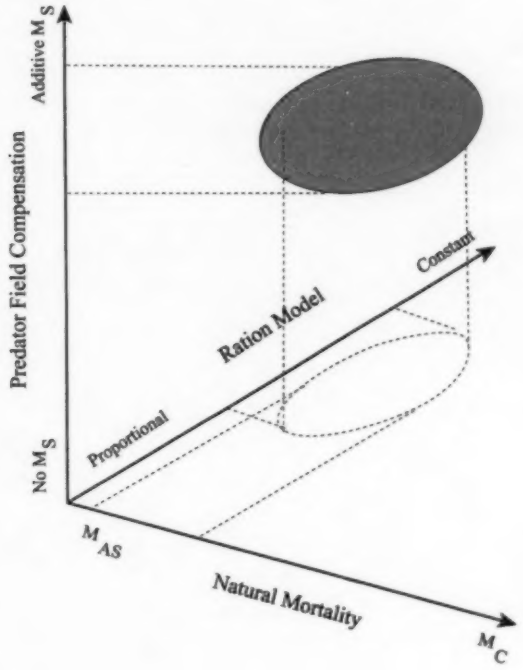
Fig. 13. A hypothetical space defined by the principal assumptions in the predation model. The ellipsoid depicts our subjectively defined region for the likely domain of the grey seal – ESS cod model.



in the model precision must await better estimates of the ADMR of grey seals.

The significance of these consumption estimates is more meaningful when they are expressed as mortality rates. A comparison of the relative magnitudes of mortality caused by fishing and grey seals provides an assessment of their relative contributions to the recently observed decline in ESS cod abundance. Under the constant ration model with residual $M = M_C$, M_S averaged about 16% of fishing mortality over the period 1970–1992, and about 11%, for the same period, under the assumption of residual $M = M_{AS}$. Similarly for the proportional ration model, the fractions were about 20 and 13%, respectively, for the two assumptions about residual M. As fishing mortality is based on reported landings rather than the actual catch from the stock, fishing mortality may have been even higher than reported here. Thus, the model results show that grey seals played a relatively minor role in the recent collapse of this fishery. This conclusion is robust to model uncertainties.

However, the larger and perhaps increasing predation mortalities seen in the 1990s raise the possibility that grey seal predation could inhibit the recovery of the ESS cod and reduce the yield from future fisheries. How likely is this possibility? The answer seems to depend very much on what we know about the magnitude of other components of M on cod, the functional form of grey seal predation, and the extent to which seal predation mortality on cod is additive. Figure 13 is a three-dimensional representation of these three principal uncertainties. To assess the importance of these uncertainties on our conclusions, we have attempted to span this hypothetical space by assuming extremes. For example, in the case of the functional form of predation, we assume that the fraction of cod in the diet is either constant (i.e., independent of cod abundance) or directly proportional to cod abundance. The axes in Fig. 13 have been arranged such that the impact is decreased as the origin is approached. Although the axes are displayed as being orthogonal, there would be a strong interdependence among them. The ellipsoid depicts our subjectively defined region for the likely domain of the grey seal – ESS cod model.

There seems to be broad agreement that the M of juvenile fish is greater than that of adults (e.g., Sparholt 1990), but less agreement or data on actual age-specific estimates for Atlantic cod. Nevertheless, it seems clear that the usual assumption of $M = 0.2$ for all ages is unlikely to hold and that estimates of seal predation mortality on ESS cod are more likely to approach those calculated under our assumption that $M = M_{AS}$. The number of cod recruits estimated from the model is very sensitive to the assumed background level of M. This in turn has a great effect on the level of mortality associated with grey seal consumption of cod and the potential impact of this predation mortality.

The amount of ESS cod eaten by grey seals depends heavily on the form of the predation model. Given the dramatic drop in the abundance of ESS cod, the proportional model predicts that grey seals would consume considerably less cod (about 4000 – 10 000 t) in 1994 than under the constant ration model (about 10 000 – 22 000 t). The proportional model also predicts little difference in short-term projections of recruitment between the model with or without explicit grey seal predation. We do not know which type of functional response best describes grey seal predation. Three types of functional responses were proposed by Holling (1959). They all increase from the origin and have an asymptotic nature. We have proposed two simpler models of functional response. The constant ration model may be thought of as type 0, as it has no slope, whereas the proportional ration model is like a type 1 except that no plateau is defined, although logically it must exist. These simpler forms were used because existing data were insufficient to fit the more complicated forms and because they represent a broad range of predator functional response.

By analogy with other vertebrate predators, there are theoretical reasons for expecting that grey seals may exhibit a type 2 or 3 functional response (Holling 1959; Peterman and Gatto 1978; Murdoch and Oaten 1975; Hilden 1988). A type 2 functional response appears to be characteristic of fish faced with a single prey, but prey switching results in type 3 responses (reviewed in Murdock and Bence 1987). The type 2 response is common in invertebrates and small mammals (Taylor 1984). Empirical studies suggest that it will be difficult to distinguish type 2 and type 3 functional responses in field data of the type that will be possible to gather for grey seals and other marine mammals. Yet these two responses can produce quite different system behaviour with respect to the impact of predation on prey populations (Peterman and Gatto 1978; Hilden 1988): a type 3 response is stabilizing whereas a type 2 response is destabilizing and is thought to be the reason why freshwater predators are often capable of eliminating prey populations (reviewed in Murdock and Bence 1987). Clearly, more research is needed on large mammalian predators.

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Recent studies (Bowen et al. 1993; Bowen and Harrison 1994) indicate that northern sand lance (*Ammodytes dubius*) is the dominant prey offshore and that pelagic species such as herring account for most of the inshore diet. Thus, grey seal population size and predation pressure may be only weakly coupled with the abundance of ESS cod such that grey seals may continue to prey on cod even when the abundance of cod is low. It is this possibility of a depensatory mechanism that could affect the magnitude and timing of the recovery of ESS cod.

We have only examined one model of the interaction between seals and ESS cod, a simple grey seal – fishery – cod model. Harwood (1983) and Beverton (1985) discuss a number of more complex models for the interactions between seals, fisheries, and prey populations that should be considered in future work. The concept of a minimum realistic model has recently emerged from modelling studies of the interactions among Cape fur seals (*Arctocephalus pusillus pusillus*), commercial fisheries, and fish species in the Benguela current (Anonymous 1991; Butterworth 1992). The concept is that attempts to evaluate the impact of seal predation on commercial fish stocks should be based on multispecies models that account for the more important species and their interactions. Clearly, the challenge is to know what to include (i.e., what is important) from potential data sources.

The grey seal – fishery – ESS cod model we have developed falls short of what is likely to be a minimum realistic model for the ESS study area. Our lack of knowledge about other predators of cod and the abundance and dynamics of other prey, especially sand lance, prohibits the development of more detailed models at this time. Young cod are eaten by fish predators on the ESS and adjacent areas (Kohler and Fitzgerald 1969; Langton and Bowman 1980), but there are no current data on the extent of this predation. In other marine ecosystems, fish predators are the major source of M (e.g., Overholtz et al. 1991; Bax 1991; Livingston 1993), particularly on young of the year fish. Thus, the magnitude of fish predation on ESS cod needs to be determined.

The impact of seal predation mortality on the dynamics of cod also depends heavily on the extent to which seal mortality is additive or is compensated for by a decrease in other sources of natural mortality. Additivity is a basic assumption of multispecies virtual population models (Sparholt 1990). However, little is known about how the components of natural mortality respond to changes in predator abundance.

Despite the difficulties and uncertainties associated with producing estimates of consumption by seals, as our results underscore, such estimates are only the first step toward an understanding of the impact of seal predation on prey population dynamics. In the context of fisheries management, estimates of consumption are of relatively little value unless they can be expressed in terms of a mortality rate. This increases uncertainty because of the imprecise nature of estimates of the size- or age-specific abundance of fish populations. Further uncertainty about the impact of seal predation mortality on the dynamics of prey populations arises because we do not know the functional relationships among or the magnitude of the other components of natural mortality. Thus, we are faced with increasing levels of uncertainty as we move from calculations of population food consumption, to estimates of predation mortality, and finally to estimates of the effect of this seal predation mortality on the dynamics of prey populations. Although important questions remain to be answered with respect to the seal predation (for example, bias in diet composition, at-sea distribution, and functional response to prey density), some of the greatest sources of uncertainty can be traced to our lack of knowledge about the age-specific pattern of natural mortality on young cod, the interactions among sources of natural mortality on cod, and the abundance and dynamics of forage fish populations such as sand lance that are shared prey of both grey seals and Atlantic cod.

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Variation in the time of spawning of Atlantic salmon (*Salmo salar*) and its relationship to temperature in the Aberdeenshire Dee, Scotland

John H. Webb and H. Anne McLay

Abstract: Time of spawning of Atlantic salmon (*Salmo salar)* in the River Dee in northeast Scotland was assessed from cumulative counts of redds made in five areas of the river system. Spawning occurred earliest at high altitude sites in the upper reaches of the river and progressively later at sites further downstream. The period of spawning at each location varied between 18 and 48 days and was more protracted at low altitude sites. Hatch occurred earlier in ova from redds in the lower altitude sites. Differences in the timing of spawning and hatch are considered in relation to water temperature. It is suggested that variation in the time of spawning is of adaptive significance and that it relates to the optimal timing of fry emergence in different parts of the river system.

Résumé : Nous avons évalué le moment de la fraye du saumon atlantique (*Salmo salar*) dans la rivière Dee (nord-est de l'Écosse) à partir de recensements cumulatifs des nids dans cinq zones du réseau de ce cours d'eau. La fraye se produisait tout d'abord en altitude, dans le cours supérieur de la rivière, puis de plus en plus tard vers l'aval. D'un endroit à l'autre, la durée de la fraye variait entre 18 et 48 jours, et elle était plus longue aux sites de faible altitude. Les oeufs des nids situés à faible altitude éclosaient plus tôt. Nous examinons les différences entre les dates de fraye et d'éclosion en fonction de la température de l'eau. Nous pensons que la variation du moment de la fraye a une signification sur le plan de l'adaptation et qu'elle est en rapport avec le moment optimal d'émergence des alevins dans les différents tronçons du réseau hydrographique.
[Traduit par la Rédaction]

Introduction

Atlantic salmon (*Salmo salar*) originating from different river systems show considerable variation with respect to growth rate, age at maturity, and the time of return migration (Schaffer and Elson 1975; Thorpe and Mitchell 1981). Much of this phenotypic diversity may be environmentally determined. However, the tendency of salmon to return to their natal rivers to spawn (Stabell 1984) would also allow for populations to evolve traits that maximize fitness in particular habitats. There is evidence that salmon home within rivers (Saunders 1967; Youngson et al. 1994) and it is postulated that local adaptation of populations may operate on broad geographical and narrower scales (Taylor 1991).

Salmon spawn in the autumn and winter months and eggs hatch and fry emerge from riverbed gravels the following spring. However, the period over which spawning takes place varies considerably, ranging from mid October to early November and from late December into January, in rivers in northern Europe (Calderwood 1900; Heggberget 1988). Water temperature is an important determinant of the length of the egg incubation period and rate of development post-hatch (Hamor and Garside 1976; Peterson et al. 1977; Wallace and Heggberget 1988) and timing of hatch and emergence are probably critical to fry survival on a site-specific basis (Bams 1969; Godin 1980; Jensen et al. 1991). Wallace and Heggberget (1988) found little evidence of differences between stocks in responses to temperature during incubation, and it is evident that, given any particular temperature regime, the date at which eggs are spawned will affect the time at which they hatch and fry emerge.

Heggberget (1988) found that the time of peak spawning of Atlantic salmon originating from rivers throughout Norway was significantly correlated with mean water temperatures between December and April but not with latitude or water flow; ovulation occurred earlier among females originating from rivers in which mean temperatures were low (<1.5°C). Temperatures decreased throughout the spawning period in all rivers studied and Heggberget (1988), together with Jensen et al. (1991), suggested a link between water temperature and spawning such that spawning is triggered at a time that will result in hatching at a specific and presumably optimal time for fry survival.

Local climatic conditions and geographic features, in particular altitude, influence thermal regimes of larger Scottish rivers. In the River Dee in northeastern Scotland, headwater tributaries are significantly cooler in the autumn, winter, and spring than those lower down (Shackley and Donaghy 1992). Radiotelemetry and conventional tagging studies have shown that adults returning to the Dee home within the river (Youngson et al. 1994) and have revealed structuring of the seasonal pattern of adult return (Hawkins and Smith 1986; Laughton and Smith 1992). To determine whether variation in the time of spawning of Atlantic salmon observed between rivers has a

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Fig. 1. The Aberdeenshire Dee, Scotland. Sites (A–E) at which spawning was monitored and the position of adult fish traps referred to in the text are indicated.

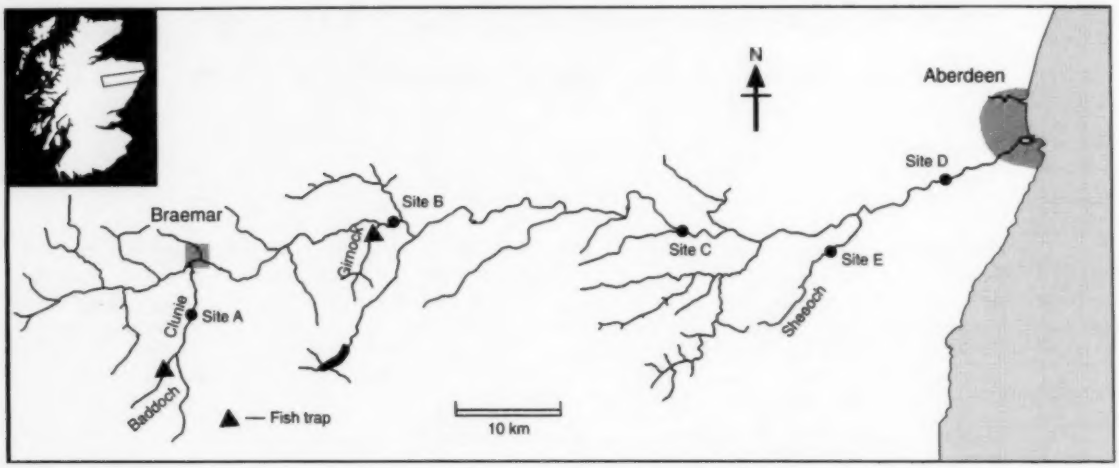
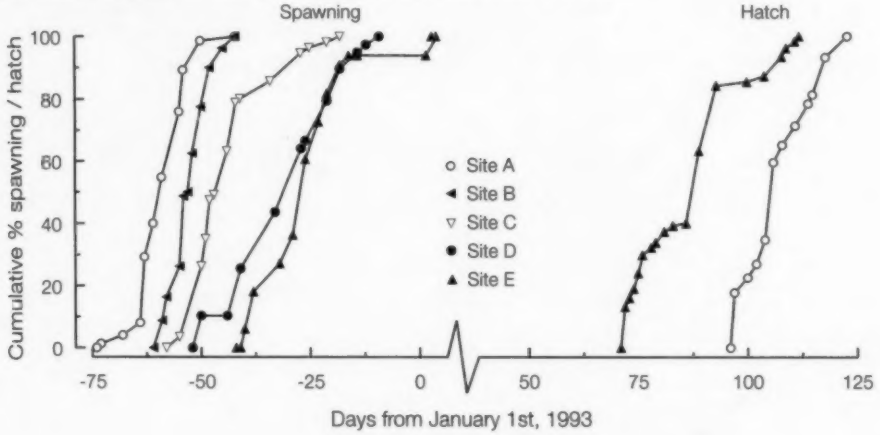


Fig. 2. Cumulative percentage spawning at sites A, B, C, D, and E and hatch at sites A and E.



within-river counterpart that is related to water temperature, we investigated the time of spawning at five sites at different altitudes in the River Dee.

Methods

Aberdeenshire Dee

The Dee rises at an altitude of about 1200 m. The main stem of the river is about 126 km long and, together with its 17 major tributaries, drains a catchment area of about 2100 km^2. The mean gradient of the river's main course is 8.9 m·km^{-1} (Maizels 1985). Areas of riverbed gravel suitable for spawning by Atlantic salmon and migratory trout (*Salmo trutta*) are found throughout the system. Spawning occurs at sites in the main stem of the river and in all accessible tributaries at a range of altitudes from the head of tide to 500 m.

On the basis of observations of the distribution of salmon made in previous spawning seasons, the following five areas (Fig. 1) were chosen for study in 1992: site A, a 1.0-km length of the water of Clunie (mean width 15.5 m), a principal upper spawning tributary that enters the Dee at Braemar 120 km from the mouth of the river at Aberdeen, altitude ~400 m; site B, a large spawning ford (~1320 m^2) traversing the bed of the main river at Polhollick, ~85 km from the river mouth, altitude ~220 m; site C, a section of the river channel (area ~1680 m^2) towards the north bank at Sluie, altitude 75 m; site D, a section (~2700 m^2) of the river channel towards the north bank of the river at Myrtle, 10 km from the river mouth, altitude 5–10 m; site E, a 1.0-km length of the Sheeoch Burn, a lower spawning tributary (mean width 5.5 m), altitude ~65 m, which enters the lower reaches river 27 km from the river mouth.

Water temperatures at sites A, B, and E before and during spawning were recorded at hourly intervals using submersible recorders (Hugrun, Iceland). Monitoring at sites A and E was continued throughout the following year.

Time of spawning

From late September 1992, the study areas were surveyed for the presence of adult salmon and for the spawning nests, or redds, in which females deposit and bury their eggs. Redds appeared as oval-shaped, excavated gravel structures, somewhat lighter in colour than the surrounding undisturbed substrate, which varied in length from

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Table 1. The timing and duration of spawning of Atlantic salmon at five sites within the River Dee, between October 1992 and January 1993.

Site	Start date	Finish date	Duration (days)	Number of redds
A	92-10-20	92-11-20	32	75
B	92-11-03	92-11-20	18	80
C	92-11-07	92-12-14	42	57
D	92-11-12	92-12-29	48	39
E	92-11-22	93-1-5	45	33

Note: Pairwise comparisons indicate that the time of spawning at each site differs significantly from all others except sites D and E.

1.0 to 3.5 m. Surveys were conducted at intervals of between 1 and 5 days by two experienced observers, wearing polarizing glasses and working from the riverbank and wading the river.

At each of the study sites, the start of spawning was recorded as the date of the formation of the first complete redd or redds. Redd positions were marked using painted boulders placed on the riverbed and the adjacent riverbank. On each subsequent visit, the numbers of newly constructed redds were recorded and their positions were also marked. Small redds, less than 50 cm in length, which were considered to have been constructed by brown trout (*Salmo trutta*), were noted but not included in counts. Any partially excavated redds were noted and those that later proved to be abandoned were excluded from counts. Surveys were continued for 21 days after the last redd was recorded at each site.

To identify large redds made by migratory brown trout that may also have spawned in the study areas, samples of 5–10 eyed ova were excavated from a proportion of the marked redd sites relocated the following spring. Redds at sites A, B, and C were partially excavated and sampled between 6 March and 21 April 1993 and those at sites D and E were done on 29 and 30 April 1993. In a few cases, ova were not recovered and samples of yolk-sac fry were obtained later in the season by electric fishing using a circular, restricted-field anode (10.5 cm diameter) designed to attract pre-emergent fry directly from spawning gravels. In total, samples were obtained from 32, 27, 23, 20, and 18 redds at sites A–E, respectively.

Samples of ova and fry were frozen and stored at –50°C. Tissue extracts were subject to electrophoresis and species identification to *S. salar* or *S. trutta* was made by assessing glucose phosphate isomerase (GPI) isozymes patterns as described by Verspoor (1988). Observations relating to redds in which the progeny of trout were identified were excluded from data analyses.

Time of hatch

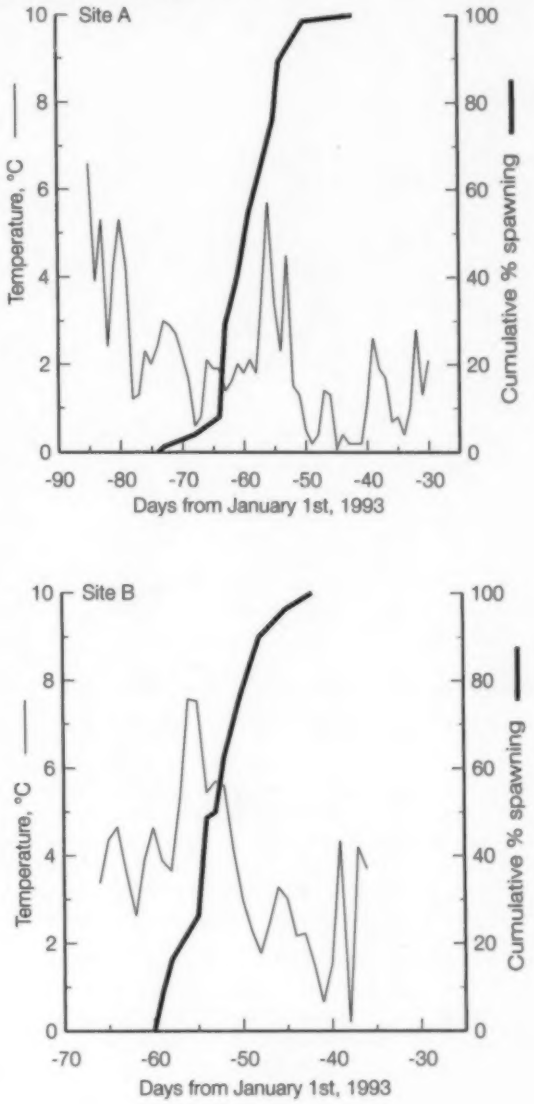
The time of hatch was assessed in groups of eyed ova excavated from 14 redds at site A and 12 redds at site E. Redds were selected to include those constructed in the early, middle, and latter part of the spawning period at each site. Groups of 50–100 ova from each redd were placed in separate, perforated (3 mm diameter holes) perspex containers lined with 1-mm plastic mesh. The containers were submerged in mesh-covered boxes in the stream at each site and were examined at intervals of between 1 and 4 days. The start of hatch was recorded for each group of ova. On each day of observation, newly hatched alevins were counted and removed. Any dead or unfertilized ova were also removed but not included in the data analysis.

Statistical analyses

The Wilcoxon rank sum test, which detects a shift in location between populations (Lehmann 1975), was used to determine whether salmon spawned earlier at one site than at another. The test was applied to each pair of sites in turn. First, the observations made at each site were assigned to one of a number of time intervals, defined by dates on which both sites were visited. For each comparison, the Wilcoxon rank sum statistic and exact two-sided probabilities were calculated using the statistical package StatXact (Metha and Patel 1991). Pairwise p values were adjusted for the number of comparisons to give the Bonferroni probability levels for differences in the timing of spawning between sites.

Median hatch dates were calculated from cumulative hatch curves plotted for each group of ova removed from redds at sites A and E, and the effects of site and time of spawning (i.e., in the early, middle, or later part of the spawning period) were examined by two-way ANOVA.

Fig. 3. Mean daily water temperatures and accumulated percentage spawning at sites A and B.



Results

Species identification

The progeny of brown trout were identified in only 4 of 120

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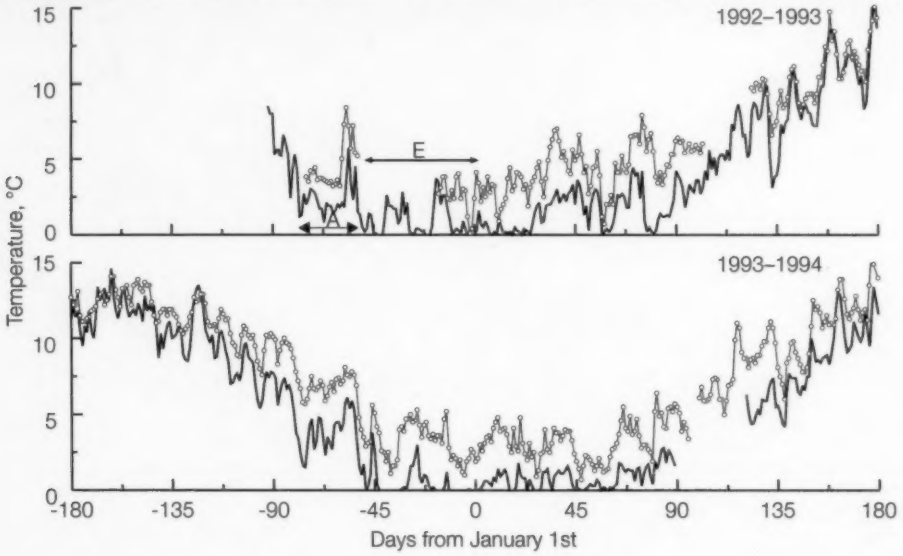
Fig. 4. Mean daily water temperatures at site A (solid line) and site E (open circles). The spawning period at each site is indicated.



redds sampled. These consisted of one redd at each of sites A and E and two redds at site C, which contained both trout and salmon.

Time of spawning and water temperatures

Spawning started earliest at the two sites in the upper reaches of the river, completed salmon redds being first noted at site A on 20 October (Table 1). The spawning period began progressively later at sites further downstream (sites C, D, and E), where it also tended to be more protracted. The last redds were observed at site E on 4 and 5 January. Variation was such that there was no overlap in the spawning periods of upper and lower tributaries, sites A and E (Table 1). The time of spawning differed between sites (pairwise comparisons, $p < 0.001$), except at sites D and E.

Once spawning had started, the numbers of redds increased more or less continuously at all study sites (Fig. 2). At sites A and B water temperatures during spawning varied between 0.8–6 and 1–8°C, respectively (Fig. 3). Because of instrument malfunction, comparable temperature records are not available for the spawning period in the lower river tributary, site E. However, longer term records of temperatures in the upper and lower tributaries (Fig. 4) indicate that considerable day to day variation in temperature is also likely to be typical of site E during the autumn. In all but the summer months, mean water temperatures at high altitude site A were lower than those at site E (Fig. 4); the mean daily differentials in water temperature at the two sites between October and April were 2.5 and 2.4°C in 1992–1993 and 1993–1994, respectively.

Time of hatch

Groups of ova excavated from redds at lower tributary site E started to hatch on 14 March 1993, 24 days earlier than those at site A. Median hatch dates for groups of ova ranged from 15 March to 19 April at site E and from 8 to 29 April at site A. For individual groups, hatching took between 2 and 21 days and was complete in all groups by 23 April and 5 May in the lower and upper tributary sites, respectively (Fig. 2). The time of hatch varied between sites and with the time of spawning within sites (Fig. 5)(ANOVA $p < 0.001$). These findings were not altered when data from a single early spawned group of eggs sampled at site E, which had an atypically late median hatching date, were excluded. The late median hatch date was probably due to oviposition at the same site by a later spawning female.

Discussion

This study has demonstrated significant variation in the time of spawning among Atlantic salmon within a river system. Variation was evident along an altitudinal gradient and shows an association with water temperature similar to that described by Heggberget (1988) in that spawning occurred earlier at high altitude sites, which typically experience lower temperatures during the winter months (Shackley and Donaghy 1992; and data this study). It has also shown that the time of spawning exerts a significant influence on hatch date. Within two of the study sites, the progeny of early spawning fish also hatched earlier, these findings being consistent under the different thermal regimes in the upper and lower tributaries.

Within the Dee, spawning was observed over periods when mean daily water temperatures ranged from 0.8 to 8.0°C. There was, however, no evidence that spawning was preceded by any particular changes in temperature or directly cued by them. Heggberget (1988) reported that mean water temperatures in Norwegian rivers varied between 1.9 and 5.2°C before spawning (peak ovulation), decreasing to 0.4 and 4.5°C during the spawning period. Baglinière et al. (1990) observed that spawning in the River Oir in northern France occurred after an increase in temperature from 6 to 9°C. Although numerous experimental studies (Lam 1983; Bye 1984) have demonstrated environmental influences on gametogenesis and spawning in

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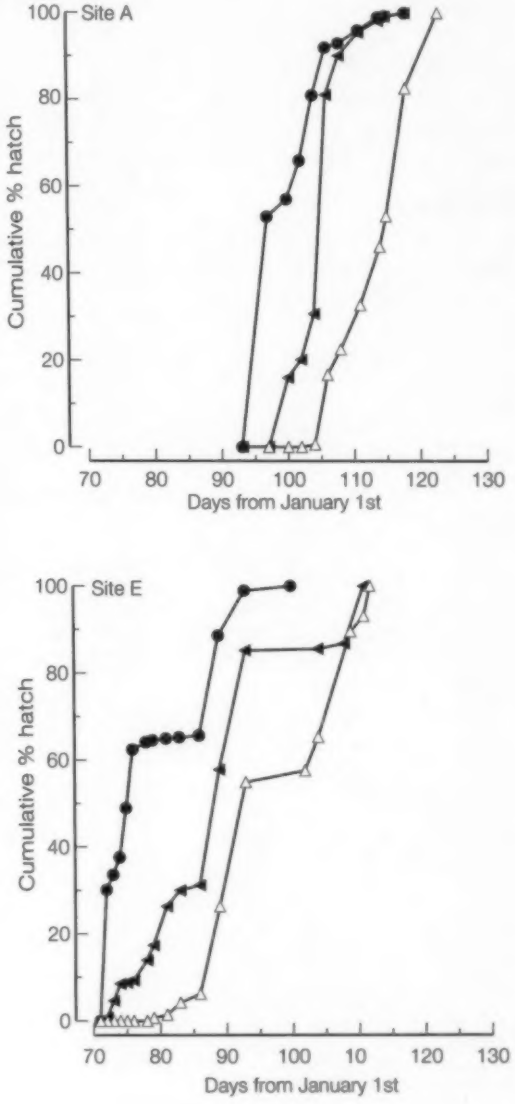
fish, Stacey (1984) noted that the salmonid ovulatory mechanism is relatively insensitive to exogenous factors and that the effects of environmental manipulation on ovulation are mediated indirectly through changes in the overall rate of ovarian development. This, and the wide range of temperatures at which spawning occurs, argues against water temperatures acting as a proximate environmental cue for ovulation or spawning.

In salmonids there is likely to be strong selective pressure on the timing of hatch and emergence both to maximize the length of the growth period and to coincide with favourable conditions of water flow, temperature, and food supply (Bams 1969; Godin 1980; Brännäs 1987; Chandler and Bjorn 1988). Heggberget (1988) suggested that variation in the time of spawning of Atlantic salmon in different rivers was linked to water temperature and the optimal timing of fry emergence. Using spawning time and environmental data, Jensen et al. (1991) modelled the time of hatch and emergence in different rivers in Norway and proposed that initial feeding was timed to avoid peak spring flows and not to occur before temperatures exceed 7–8°C. In Pacific salmonids, variation in the time of spawning has also been related to water temperature during the incubation period, with early spawning fish being found in areas in which overwintering temperatures are relatively low (Sheridan 1962; Burger et al. 1985; Brannon 1987). However, disparity in spawning times and compensation in rates of development at low temperatures act to stabilize times of emergence and stocks differ in their response to temperature such that they converge towards a common emergence date (Brannon 1987). In the Dee, the progeny of later spawning fish in the lower reaches of the system hatched earlier than those in the upper tributary and, in contrast to the determined spawning period, there was some overlap in time of hatch at the two sites. If variation in the time of spawning in the Dee is related to the optimal time of hatch and emergence, our findings suggest that this differs within the river. That spawning occurs over a more protracted period at lower altitude sites within the Dee may be related to environmental variability or to other factors that favour fitness of fry emerging over a more extended period.

Brannon (1987) considers spawning time to be a genetic characteristic of stocks that has been selected to assure optimal emergence timing. Studies of heritability of spawn date in other salmonids implicate additive genetic and maternal influences on the trait (Siitonen and Gall 1989; Danzmann et al. 1994). Given the evidence of reproductive isolation and homing of fish within the Dee (Youngson et al. 1994), it is considered that spawning time is a population-specific characteristic. To establish whether differences have adaptive relevance in relation to the timing of fry emergence would require demonstration of differential fitness in the progeny of fish spawning at different times in different areas. However, that they are persistent and reflect inherent differences in the rate of reproductive development and time of ovulation, rather than responses to water temperature, is supported by observations made at fish-trapping facilities on two tributaries near sites A and B. Since comparative studies began in 1988, fish captured in the upper tributary, near site A, have been found to ovulate and spawn earlier than those captured further downstream at the trap near site B (D.W. Hay, unpublished data). Water temperatures during October and November at the lower trap site show considerable year to year variation (Youngson et al. 1983), which suggests that temperature would not be a sensitive indicator of date.

Fig. 5. Cumulative percentage hatch in groups of ova excavated from redds formed in the early (solid circles), middle (solid triangles), and late (open triangles) phases of the spawning periods at sites A and E.



Among anadromous salmonids, the probable link between the tempo of adult sexual maturation, return migration, and time of spawning has not been fully explored. Within the Dee, there is evidence that fish entering the river before May, so-called spring-run fish, home to and spawn in the upper reaches of the catchment whereas later running fish spawn lower down (Hawkins and Smith 1986; Laughton and Smith 1992). Differences in the time of spawning of salmon in the Dee thus parallel differences in their time of river entry. Variation in the

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time of spawning observed may be one manifestation of genetic differentiation with respect to the rate of reproductive development, which is also evident as differences in the time of the onset of sexual maturation and variation in the time of river entry.

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Temporal tracking of a strong cohort in the population of a coral reef fish, the coral trout, *Plectropomus leopardus* (Serranidae: Epinephelinae), in the central Great Barrier Reef, Australia

Garry R. Russ, Dong C. Lou, and Beatrice P. Ferreira

Abstract: We document a strong year-class in the population of coral trout (*Plectropomus leopardus*) in the central Great Barrier Reef, Australia. From 1990 to 1993 samples of coral trout were collected at two coral reefs closed to fishing since 1987, and at two reefs open to fishing. On the closed reefs the age structure was dominated by a strong cohort that settled in early 1984 and that accounted for 54, 45, 42, and 36% of the experimental hook and line catches in 1990 to 1993, respectively. A similar strong cohort was not obvious on the fished reefs close by, possibly owing to small sample sizes. This is the first time a dominant age-class has been tracked over time for any coral reef fish by examination of age structures. The results demonstrate that strong interannual fluctuations in recruitment can affect the local abundance of coral trout populations substantially and that strong recruitment events can be retained in the age structure of coral trout populations. Furthermore, because age of recruitment to the fishery is approximately 3 years, these results suggest the possibility that recruitment strength may be predicted up to 3 years in advance using yearly surveys of juvenile fish.

Résumé : Nous avons étudié une forte classe d'âge dans la population de mérou étoiles bleues (*Plectropomus leopardus*) du centre du récif de la Grande Barrière, en Australie. De 1990 à 1993, nous avons prélevé des échantillons de ce mérou sur deux récifs de coraux fermés à la pêche depuis 1987, et sur deux récifs où la pêche était ouverte. Sur les récifs fermés, la structure d'âge était dominée par une forte cohorte qui s'est établie au début de 1984 et qui a représenté 54, 45, 42 et 36% des captures expérimentales effectuées à l'hameçon de 1990 à 1993, respectivement. La présence d'une cohorte aussi forte n'est pas apparue sur les récifs voisins où la pêche était ouverte, ce qui peut être dû à la faible taille des échantillons. C'est la première fois qu'on peut suivre chez des poissons des coraux une classe d'âge dominante au fil du temps en examinant les structures d'âge. Les résultats démontrent que de fortes fluctuations interannuelles du recrutement peuvent avoir un effet notable sur l'abondance locale des populations de mérou étoiles bleues, et que les épisodes de recrutement importants peuvent rester présents dans la structure d'âge des populations de cette espèce. En outre, étant donné que l'âge du recrutement à la pêche est de 3 ans environ, les résultats indiquent que des recensements annuels des poissons juvéniles pourraient permettre de prédire jusqu'à 3 ans d'avance l'effectif du recrutement.
[Traduit par la Rédaction]

Introduction

Since the turn of the century, fisheries scientists have known that the fluctuations in yields of important food fish of temperate species were influenced strongly by variable year-class strength (Hjort 1914, 1926; Gulland 1982; Sissenwine 1984; Rothschild 1986). The bioeconomic consequences of fluctuating yields caused by variable year-class strength are well known, as are the collapses of stocks where fishing levels did not make sufficient allowance for the possibility of repeated recruitment failure (Laevastu and Favorite 1988). The combination of high fecundity and high and variable rates of larval mortality may result in large variations in the strength of replenishment of the next generation (Underwood and Fairweather 1989). Variations in recruitment strength can significantly affect the dynamics and demography of fish populations (Nikolskii 1969; Sissenwine 1984; Rothschild 1986) . Analysis of the age structure of exploited fish stocks in the Northern Hemisphere has shown that consecutive cohorts of juveniles may not contribute equally to population biomass (Nikolskii 1969). In several fisheries, it has been demonstrated that year-class strength is largely determined very early in the life history and that all age groups subsequently remain roughly in proportion to the annual abundance of the 0+ cohort (Gulland 1982).

In contrast to the long history of research on the fluctuations in year-class strength of temperate fisheries of the Northern Hemisphere (Hjort 1914, 1926; Sissenwine 1984; Rothschild

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1986), studies of such variation in tropical fish stocks, in particular stocks of fish on coral reefs, began relatively recently around the mid 1970s (see reviews by Ehrlich 1975; Sale 1980; Victor 1983; Doherty and Williams 1988; Doherty 1991; Sale 1991). Furthermore, recognition and routine use of annual banding patterns in the otoliths of coral reef fishes have occurred relatively recently (e.g., Manooch 1987; Polovina and Ralston 1987; Fowler 1990; Ferreira and Russ 1992; Lou 1992) precluding previous use of age-structure data. Retention of fluctuations in year-class strength in the age structure of a coral reef fish has recently been reported for the damselfish *Pomacentrus moluccensis* (Doherty and Fowler 1994). There has been little published research on the fluctuations in year-class strength of commercially important fish on coral reefs (but see Doherty et al. 1994) even though fishing is one of the most important human exploitative activities on coral reefs (Munro and Williams 1985; Russ 1991).

The coral trouts of the genus *Plectropomus* Oken are members of the serranid subfamily Epinephelinae, commonly known as groupers. They support important fisheries throughout the tropical and subtropical regions of the world (Ralston 1987). On the Great Barrier Reef (GBR), the common coral trout, *Plectropomus leopardus* (Lacepede 1802), is the most abundant species in the genus (Randall and Hoese 1986) and it is usually the primary target of recreational and commercial fishers (Craik 1989; Trainor 1991). The Queensland commercial line-fishing fleet takes a total annual catch of about 4000 metric tons (t) of reef and pelagic species. The coral trout comprises the largest single component of this catch (over 30%) with around 1200 t caught annually (Trainor 1991). Because of its importance, the coral trout has been the subject of many studies investigating the abundance and size structure of populations under different fishing conditions on the GBR (see Williams and Russ 1994, for review). Most of these studies were conducted using underwater visual census (UVC) techniques to examine the size structure of populations (e.g., Craik 1981; Ayling and Ayling 1984, 1986). Only one study has investigated the short-term population structure of coral trout in the context of age composition (Ferreira and Russ 1995). On the basis of 2 years of age structure data at four reefs on the GBR, Ferreira and Russ (1995) reported a strong year-class in the population of coral trout on reefs closed to fishing. However, the duration of monitoring (2 years) was relatively short in relation to the maximum potential life-span of coral trout (14 years: Ferreira and Russ 1994). The present paper extends the initial sampling reported by Ferreira and Russ (1995) to confirm the long-term persistence of a strong cohort in the age structure, and stresses the long-term impact of good recruitment to the fishery. There has been no research on the long-term patterns of age structure of coral trout populations. The age and growth of coral trout have been recently validated (Ferreira and Russ 1992, 1994), making it possible to use age as an indicator of changes in population structure under different levels of fishing pressure and through time.

The objectives of this study were to investigate year-class strength in the populations of coral trout on reefs zoned to either prevent or allow fishing on the GBR by monitoring age composition. The specific question is, can variation in year-class strength be retained and tracked in the age structure of coral trout populations?

Materials and methods

Study sites and sampling methodology

Four midshelf reefs off Townsville, in the central GBR, Australia, were sampled in this study. These reefs were Glow, Yankee, Hopkinson, and Grub, approximately 70 km northeast of Townsville (Fig. 1). Two reefs, Grub and Hopkinson, were located in General Use Zones, and were open to line and spear fishing, while the other two, Glow and Yankee, were located in National Park Zones, and had been closed to fishing since September 1987.

From late June 1990 to May 1994, a total of 945 coral trout were collected by line fishing (Table 1). On each sampling trip, four amateur (recreational) line fishers fished at each reef for approximately 4 daylight hours. The fishing crew was relatively consistent in composition, with overall fishing ability maintained as consistent from trip to trip as possible. The sampling vessel for 1990–1992 was the 10.1-m *Challenger* whereas in 1994 the 10.5-m *Amanda Jane* was used.

Fishes sampled were measured to the nearest millimetre (fork length) and weighed to the nearest 10 g while fresh. The heads of fishes were frozen immediately upon collection and processed for otolith analysis later in the laboratory.

Otolith preparation

To determine age of fish, sectioned sagittae were read following the methodology described by Ferreira and Russ (1994). The sagitta was prepared for reading by first embedding in epoxy resin (Spurr 1969) and then sectioning transversely through the core with a Buehler Isomet low-speed saw. Sections were then mounted on glass slides with Crystal Bond 509 adhesive, ground on 600- and 1200-grade sandpaper, polished with 0.3-mm alumina, and then examined under a dissecting microscope at 40× magnification with reflected light on a black background. Annuli were counted from the nucleus to the proximal surface of the sagitta along the ventral margin of the sulcus acousticus. Double blind counts were made on all otoliths. If there was disagreement in the number of otolith bands between the replicate counts the readings were repeated once and if the counts still differed, the otolith was discarded.

Coral trout recruitment occurs in the first few months of the year (Doherty 1991; Doherty et al. 1994), so the birth date was assigned as the 1st of January. Opaque zones are formed once a year, from July to November (Ferreira and Russ 1994), and were counted only when there was further deposition of a translucent zone, i.e., from December onward. Therefore, the number of rings corresponded to the real age of fish. Furthermore, the outermost full opaque zone of otoliths of coral trout collected from March to May 1994 was actually deposited in 1993. Thus, these samples were termed the 1993 sample in this paper.

Results

The age-frequency distributions of coral trout samples from the closed reefs indicated a strong age-class dominating the populations at Glow and Yankee from 1990 to 1993 (Fig. 2). That age-class was 6, 7, 8, and 9 years old in 1990, 1991, 1992, and 1993, respectively, and comprised 38.8–53.4% of the total catches at Glow and 35.5–54.4% at Yankee. This observation suggests that strong selection towards one age by line fishing gear, or bias in the determination of age, was unimportant. This strong year-class, however, was not evident on the open reefs except for a suggestion of it on Hopkinson (Fig. 3). Sample sizes were, however, very small for the open reefs (see Table 1).

This strong age-class (cohort) settled onto the closed reefs at the beginning of 1984 (see Ferreira and Russ 1995). As Glow and Yankee have been closed to fishing since 1987, and

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Fig. 1. Location of sampled reefs: Glow and Yankee (closed to fishing) and Grub and Hopkinson (open to fishing), on the Great Barrier Reef, Australia.

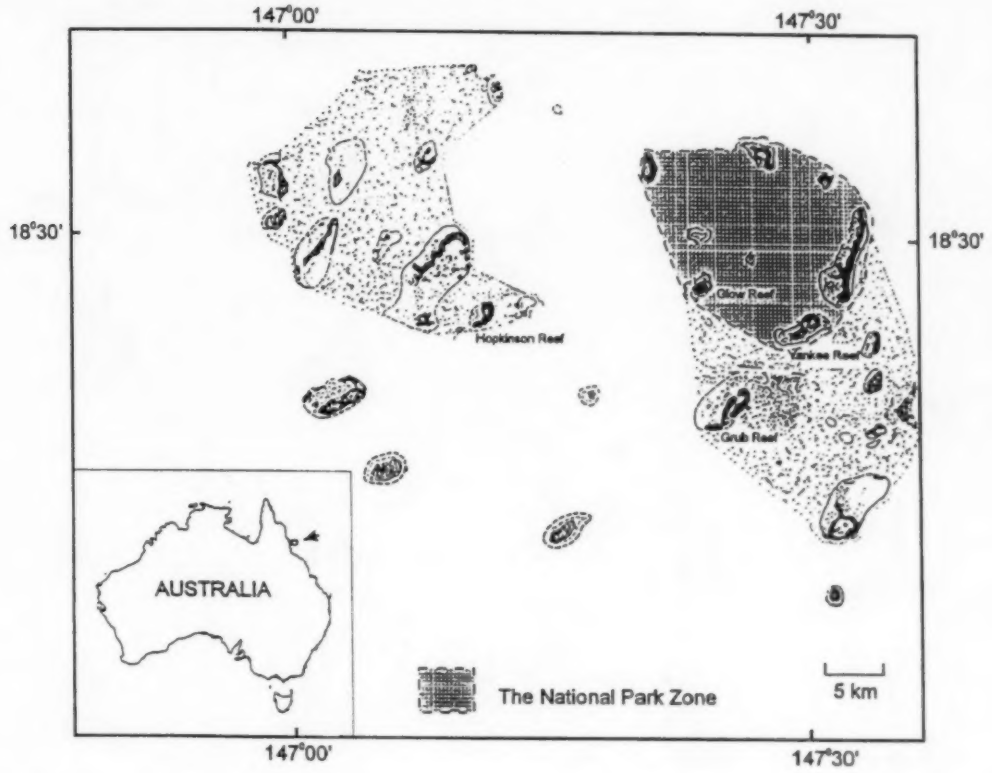

Table 1. Sampling dates and number of coral trout, *Plectropomus leopardus*, collected in each sampling trip.

		Closed		Open	
Month	Year	Glow	Yankee	Grub	Hopkinson
June–July	1990	52	18	9	14
September–October	1990	50	42	11	18
June–July	1991	74	54	14	29
September–October	1991	24	11	15	15
August	1992	—	33	24	—
October	1992	62	—	—	23
March	1994	48	—	—	25
April	1994	—	57	22	—
May	1994	52	71	24	54
Total		362	286	119	178

Note: —, zero effort.

age of recruitment to the fishery is approximately 3 years (Ferreira and Russ 1995), the individuals settling onto Glow and Yankee in 1984 were protected from fishing for most of their lives.

By combining the age frequency data from the two closed reefs, it was clear that the 1984 year-class dominated the total catch over all 4 sampling years (1990–1993) and comprised >50% of the catch in 1990 even though 14 year-classes were represented in the catches. The strength of this year-class decreased gradually from 54% of the catch in 1990 to 36% in 1993 (Fig. 4*a*). In contrast, the combined length frequencies from the closed reefs showed no obvious modal progression from 1990 to 1993, and modal lengths of catches from these 4 years overlapped substantially (Fig. 4*b*). This reflects the large variability in size at age of this species (Ferreira and Russ 1994). The size range for a given age in this strong year-class was 33–51, 33–53, 35–55, and 36–55 cm in 1990, 1991, 1992, and 1993, respectively (Fig. 5). Thus, a single age-class often contributed substantially to multiple length peaks in any particular year of sampling.

Discussion

This paper provides the first direct evidence that the dynamics of fish populations of commercial and recreational significance on coral reefs may be influenced strongly by differences in year-class strength of recruits and that such differences are preserved in the age structure. The age structure of coral trout populations from the closed reefs in this study was dominated by the presence of a strong cohort, with this strong cohort dominant at two coral reefs separated by 10 km (Fig. 2). The occurrence of strong cohorts is well documented in the commercial catches of many temperate species (Hjort 1914, 1926; Sissenwine 1984; Rothschild 1986) and has recently been confirmed in a species of tropical damselfish (Doherty and Fowler

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Fig. 2. Age distribution of coral trout for the closed reefs in each sampling year. The percentage of the catch accounted for by the strong year-class is shown for each year.

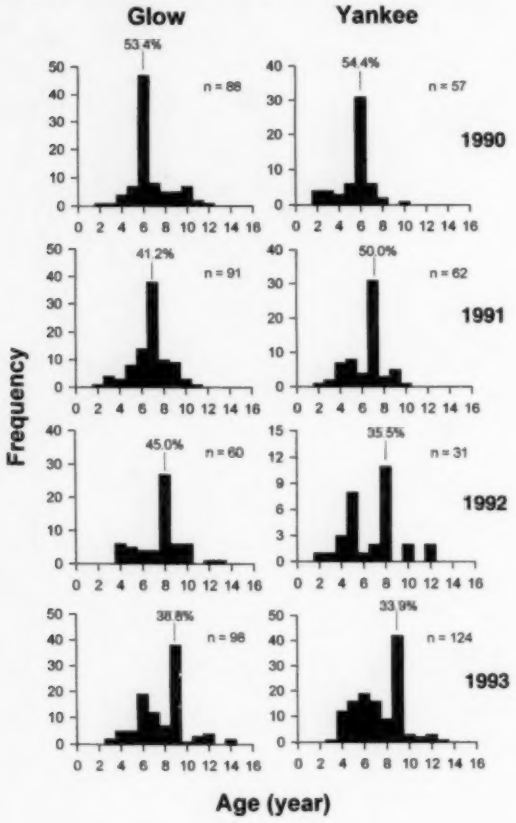
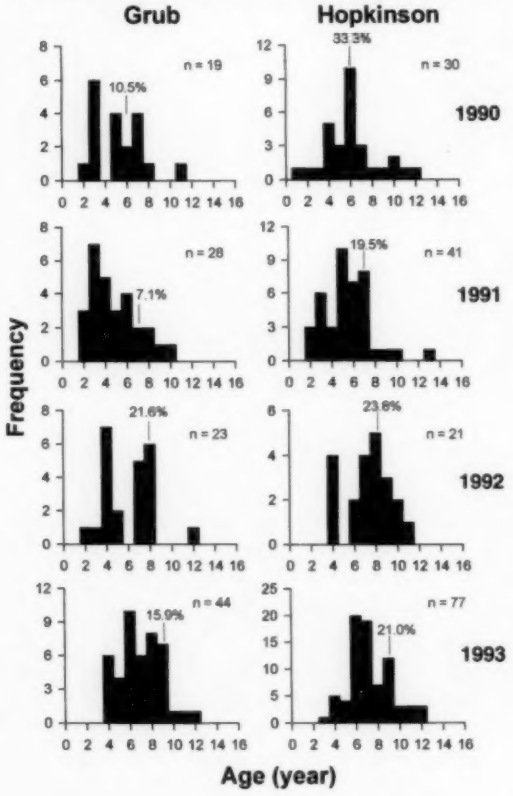


Fig. 3. Age distribution of coral trout for the open reefs in each sampling year. The percentage of the catch accounted for by 6, 7, 8, and 9 year olds is shown for the years 1990 to 1993, respectively.



1994). Year-class strength in both temperate and tropical species has been linked to early life-history processes (e.g., Hjort 1914; Sissenwine 1984; Doherty and Fowler 1994). Furthermore, the possibility that recruitment variability is a major factor influencing both the distribution and local densities of coral reef fishes has been recognized for a number of years (Williams 1980; Doherty 1981, 1983; Victor 1983; Doherty and Williams 1988; Doherty 1991; but see also Shulman and Ogden 1987; Jones 1991). Subsequently, Doherty and Fowler (1994) showed that for the common tropical damselfish *Pomacentrus moluccensis*, age structures preserved annual variations in recruitment over at least a 10-year period, providing empirical evidence of a strong effect of recruitment history on subsequent year-class strength. Similarly, for the commercially important coral trout, good recruitment appears to persist in the age structure of populations as a dominant age-class over time.

It must be acknowledged that in the absence of data on the abundance of newly settled 0+ coral trout on the two closed study reefs in 1984 relative to other years and in the absence of any data on variations in postsettlement mortality rates, the existence of a dominant age-class from 1990–1993 cannot be shown unequivocally to be the result of variability in larval settlement to the reefs. However, it is difficult to envisage a mechanism that would have resulted in a substantial reduction in postsettlement mortality rate specifically of the 1984 year-class (e.g., reduced predation; see Jones (1991) and Hixon and Beets (1993) for recent reviews of the role of postrecruitment processes on reef fish) and difficult to explain how such a mechanism may operate on a spatial scale larger than a single reef. Given the wealth of evidence that variations in year-class strength are often determined very early in the life history of marine teleosts with a planktonic larval phase (Hjort 1914, 1926; Gulland 1982; Sissenwine 1984; Rothschild 1986) and evidence that large variations in abundance of 0+ fish are often retained in the age structure of the population (Nikolskii 1969; Doherty and Fowler 1994), a disproportionately high abundance of 0+ coral trout on the two closed reefs in 1984 is the most parsimonious explanation for the result shown in Fig. 2.

Sample sizes were so small on the open reefs that a clear indication of the strong year-class was difficult to detect, even if it was present. There is evidence for the possibility of strong recruitment pulses of reef fishes occurring concurrently on midshelf reefs separated by up to 10–30 km off Townsville (Doherty and Williams 1988; Williams 1991). The age-structure data for the two closed reefs provide circumstantial evidence in support of pulses of recruitment being synchronous

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Fig. 4. Percentage of catch by number of coral trout from the two closed reefs over 4 years by age and by fork length.

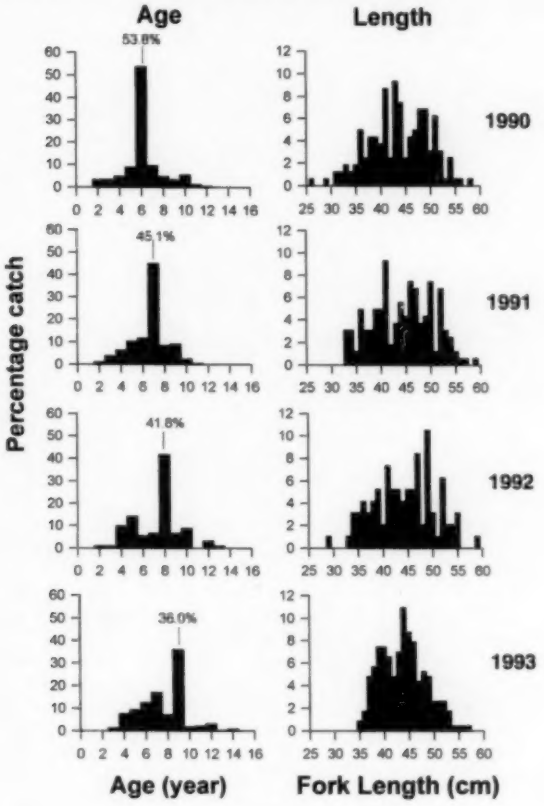
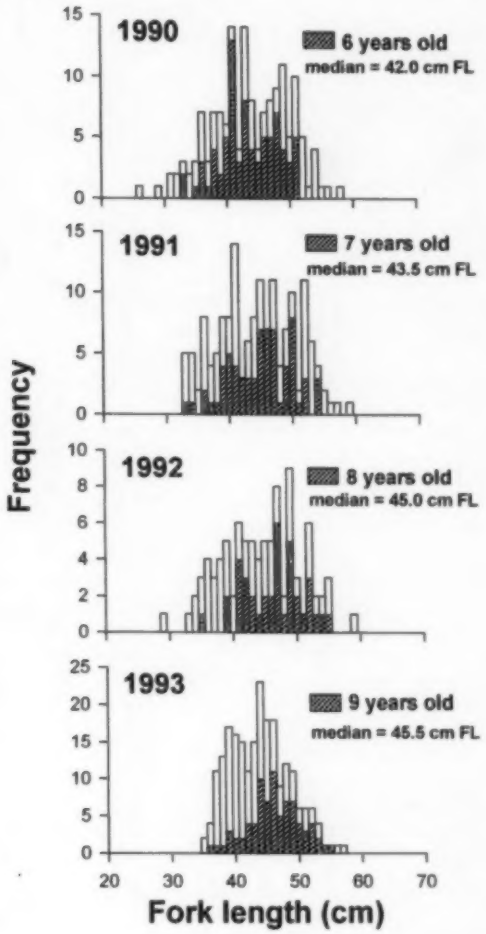


Fig. 5. Size distribution of coral trout for the two closed reefs in each sampling year. The shading shows the strong year-class. FL, fork length.



on reefs at least 10 km apart. If the strong cohort did settle onto the open reefs as well, fishing may have reduced its abundance relative to other age-classes in the population. However, this would imply the unlikely scenario of the strong year-class somehow being more vulnerable to line-fishing gear than the other year-classes.

It is unlikely that a strong cohort in the populations of relatively long-lived tropical fish with high variations in size at age could be detected by examining length-frequency distributions. Recent research has suggested the possibility of different growth processes within a population with resulting age-specific differences in fishing mortality (Parma and Deriso 1990) and natural mortality (Mulligan and Leaman 1992). The large variability in size at a given age of the coral trout (Ferreira and Russ 1994; the present study; see also Ferreira and Russ 1992) suggests the occurrence of substantial individual variability in growth. The reliability of methods of growth estimation such as length-frequency analysis techniques used for tropical fish populations is adversely affected by this kind of variation (Sainsbury 1980). Early attempts to determine growth rates and infer ages of *Plectropomus leopardus* used length-frequency data (Goeden 1978; Pauly and Ingles 1981). In fact, the species is still used in modern fisheries textbooks as an example where length-frequency samples may contain reliable estimates of growth rates (e.g., Hilborn and Walters 1992). Pauly and Ingles (1981), using just two length-frequency samples derived from Goeden (1978), estimated growth characteristics very close to estimates now available from size at age data. However, Fig. 5 of the present paper questions the suggested assignment of ages to length peaks in Goeden's (1978) original data set (see Fig. 13.4 of Hilborn and Walters 1992 for a redrawn version of the figure). The results of the present study stress the importance of attempting to collect validated age frequencies to assist in analysis of the dynamics of exploited fish populations in the tropics. The use of otolith weight as a predictor of fish age may provide a potentially cost-effective method of reconstructing age-frequency distributions of long-lived tropical species (Worthington et al.1995).

Because the abundance of coral trout populations may occasionally be dominated by a strong cohort (one cohort accounting for 36–54% of catch in numbers in the present study), knowledge of recruitment strength may permit prediction of

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abundance (at potential harvest) of coral trout several years in advance. If the abundance of coral trout populations can be influenced strongly by interannual fluctuations in recruitment, as demonstrated in the present study, this finding has important implications for the zoning strategy of the Great Barrier Reef Marine Park. One very practical aspect of these results for fisheries management is that a very large settlement of coral trout in any particular year (detected by, say, light traps or visual surveys of newly settled juveniles; see Doherty et al. (1994) who related abundance of coral trout larvae competent to settle to later abundance of 0+ fish) is likely to be followed 3 years later by a very large recruitment to the fishery. If spawning stocks were reduced to unacceptably low levels, a management agency would have 3 years after a good settlement to close a larger than average number of reefs to allow a pulse build up of spawning stock biomass. If data indicating which reefs were good sources of larvae were eventually to become available, such "source reefs" could be specifically chosen for such closures. Russ and Alcala (1996), on the basis of empirical studies and simulations, stressed the importance of strength and timing of recruitment in determining the pattern and rate of build up of biomass of large predatory fish following the closure of coral reefs to fishing.

In conclusion, a very strong cohort dominated the age structure on two reefs closed to fishing (i.e., 6, 7, 8, and 9 year olds in 1990, 1991, 1992, and 1993 respectively). This strong cohort was not obvious on fished reefs close by, although this may have been due to small sample sizes on open reefs. The strong cohort accounted for 36–54% of experimental catches, suggesting that strong cohorts may contribute substantially to catches. The fishery resource of coral trout on the GBR could perhaps be predicted by the survey of larvae competent to settle or newly settled juveniles, as the abundance of coral trout may be influenced substantially by the presence of a strong cohort. Use of size structures to analyze the dynamics of stocks of coral trout likely will fail to detect important population characteristics. Therefore, age determination should be a routine component in the assessment and management of coral trout populations.

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Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration

Jacqueline J. Dockray, Scott D. Reid, and Chris M. Wood

Abstract: Juvenile trout (*Oncorhynchus mykiss*) were exposed to a simulated global warming – acidic water scenario over a 90-day summer period (control temperature range 13–24°C). The addition of 2°C to the fluctuating summer cycle of inshore Lake Ontario and H_2SO_4 to synthetic soft water resulted in four treatments: control, acidification of control, simulated global warming alone, and global warming plus acidification. The twice-daily feeding regime raised metabolic rates to ~75% of $\dot{M}o_2$(max). Large increases (from 4.5 to 11.5%) in whole-body lipid, smaller increases (from 12.0 to 15.5%) in protein, and compensating decreases in water content (from 77 to 71%) occurred in all treatments over time. The addition of 2°C resulted in depressed appetites and growth, particularly after the period of peak temperature (days 60–90; 26°C). Metabolic rate and nitrogenous waste excretion were also depressed. Overall, exposure to low pH resulted in increased appetites and growth, the increase of 2°C reduced gross energy intake and increased fecal energy losses, and exposure to low pH resulted in increased energy intake and gain and better conversion efficiency. The lack of ionoregulatory disturbance in trout chronically exposed to pH 5.2 suggested that dietary NaCl may have compensated for branchial ion losses.

Résumé : Des truites juvéniles (*Oncorhynchus mykiss*) ont été exposées à une simulation de réchauffement global et d'acidification de l'eau pendant une période de 90 jours en été (plage de température témoin de 13–24°C). Le relèvement de 2°C du cycle fluctuant de la température estivale des eaux côtières du lac Ontario et l'ajout de H_2SO_4 à de l'eau douce de synthèse ont servi à pratiquer quatre traitements : témoin, acidification du témoin, simulation de réchauffement global seulement, et réchauffement plus acidification. Le régime d'alimentation biquotidien a fait monter le métabolisme à 75% de $\dot{M}o_2$(max). De fortes augmentations (de 4,5 à 11,5%) des lipides du corps entier, des augmentations plus faibles (de 12,0 à 15,5%) des protéines et des baisses compensatoires de la teneur en eau ont été observées dans tous les traitements (de 77 à 71%) au fil du temps. Le relèvement de 2°C a causé une diminution de l'appétit et de la croissance, particulièrement après le pic de température (jours 60 à 90; 26°C). Le métabolisme et l'excrétion de déchets azotés ont également baissé. Globalement, l'exposition à un pH faible a occasionné une augmentation de l'appétit et de la croissance. Le relèvement de 2°C a réduit l'absorption d'énergie brute et accru les pertes d'énergie par les fèces, tandis que l'exposition à un faible pH a causé une augmentation de l'absorption et du gain d'énergie, et une meilleure efficacité de conversion. L'absence de perturbation de la régulation ionique chez les truites chroniquement exposées à un pH de 5,2 permet de penser que le NaCl contenu dans l'aliment peut avoir compensé les pertes ioniques au niveau branchial.
[Traduit par la Rédaction]

Introduction

The Intergovernmental Panel on Climate Change (IPCC; 1991) has forecast that with a doubling of atmospheric CO_2, which will occur over the next 50 to 100 years if current production levels continue, and increases in other "greenhouse" gases, such as methane, nitrous oxide, and chlorofluorocarbons, global air temperatures may increase 1.3–4.5°C.

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Increased air temperatures will result in increased temperatures of freshwater bodies, which will directly affect the fisheries associated with these habitats. Regier and Meisner (1990) discuss the effects of increased temperature on water quantity and quality in the Great Lakes using an iterative assessment process. Schindler et al. (1990) described an increase in air temperature at the Experimental Lakes Area in northwestern Ontario of 2°C over the last 20 years and provided data indicating that evaporation has increased, precipitation has decreased, and consequently, water renewal rates have decreased. These changes have directly affected stenothermal fish species by decreasing available cold water habitats. From these observations, they concluded that fresh waters should be considered in major global change programmes because of the fisheries' importance as a food source and their scarcity worldwide.

Anthropogenic production of pollutants has many other environmental impacts that should be considered in concert with projected climate change. For example, the burning of coal and oil and the smelting of metallic ores result in the production of

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sulphur dioxide (SO_2), and internal combustion engines produce nitrogen oxides (NO_x). Release of SO_2 and NO_x into the atmosphere results in acid precipitation, which has decreased lake pHs by between 0.5 and 1.5 units in many softwater lakes in North America and Europe over the last 140 years (Kemp 1994). Particularly sensitive lakes, with alkalinities below 50 μequiv·L^{-1}, include those found in the eastern provinces of Canada (Schindler 1988). In Ontario, lakes found on the Canadian Shield have a highly resistant granitic base, which results in low water-buffering capacity. Acidification of these lakes has been extensively studied by Scheider et al. (1979), Schindler et al. (1980), and Kelso et al. (1982, 1986), and the deleterious effects on fish have been described by Beamish et al. (1974*a*, 1975), Kelso and Gunn (1984), and Kelso et al. (1986, 1990).

Because fish are poikilotherms, their body temperature is set by the external temperature of the water they inhabit. Of all environmental factors that affect fish, temperature is considered to be the ecological master factor (Brett 1971) and has direct influence on metabolic rates, feeding rates, and activity levels. Metabolic rate, as measured by oxygen consumption, is a composite measure of the cost of living for a fish in a particular environment. Important costs include maintenance metabolism, energy expended in feeding, nutrient assimilation, subsequent waste excretion, growth, and locomotion. All of the latter requirements for living have a requisite cost associated with them, the amount of which is set by temperature.

In fish, exposure to low pH has been shown to either reduce growth or have no effect on it (Mount 1973; Leivestad et al. 1976; Menendez 1976; Jacobsen 1977). Decreased growth may be a consequence of decreased feeding under acid conditions (Beamish 1972; Swarts et al. 1978). Low pH has also been shown to increase resting oxygen uptake ($\dot{M}O_2$), indicating increased metabolic cost, and decrease critical swimming speeds (U_{crit}), thereby reducing the scope for activity (Butler et al. 1992).

Protons exert their toxic effect at the gills by disturbing electrolyte balance. Ionoregulatory failure and subsequent fluid shifts, hemoconcentration, and circulatory collapse are the result of lethal levels of low pH (Milligan and Wood 1982; Wood 1989). Chronic sublethal levels of low pH, however, have been shown to result in stabilization of electrolyte balance and blood plasma parameters (Audet et al. 1988), and recovery of whole-body [NaCl] has been shown to occur in one study (Wilson et al. 1994*a*). The physiological changes associated with the original ionoregulatory disturbance, and adjustment to the new conditions, are considered deleterious and do not result in acclimation to more severe acid stress in rainbow trout (Audet and Wood 1988).

The physiological adjustments required by fish exposed to low pH and the increased rate of physiological processes caused by higher temperatures presumably would result in increased metabolic costs. Our goal in the present study was to assess the effects of chronic exposure to sublethal low pH (5.2) and elevated (+2°C) summer water temperatures on metabolic costs. The experiment was carried out over the summer (June–September 1993), when the summer peak temperature occurred, using juvenile rainbow trout (*Oncorhynchus mykiss*) that were fed to satiation. A suite of metabolic cost indicators was measured, including appetite, growth, metabolic rate, and the partitioning of food energy as protein, lipid, and carbohydrate. Other potential indicators of increased metabolic cost and stress that were monitored include whole-body and plasma ion levels, hematocrit, plasma protein, and lactate levels.

Materials and methods

Animal holding

Experiments were conducted on 1146 juvenile rainbow trout (2–3 g), transported from Rainbow Springs Hatchery, Thamesville, Ont., on April 19, 1993. At McMaster University, they were placed in a 400-L polypropylene tank and acclimated to and maintained in continuously flowing Hamilton dechlorinated tap water ($[Ca^{2+}] = 1.95 \pm 0.22$, $[Na^+] = 0.556 \pm 0.33$ mequiv·L^{-1}; pH = 7.4–7.8) at 11°C for 1 week after arrival. The water hardness was reduced gradually over the 2nd week by increasing the proportion of artificially softened water generated by reverse osmosis (Anderson Water Conditioning Equipment, Dundas, Ont.). When the desired water quality was achieved, the trout were acclimated for another 7 weeks. Fish were fed ~4% (dry weight of food) of their dry body weight·day^{-1} with Zeigler Trout Starter No. 3 (water, 11.66%; protein, 50%; fat, 15%; fibre, 2%; sodium, 0.50%; calcium, 2.30%; phosphorus, 1.80%) and maintained on this diet for the 8-week acclimation period before the start of the experiment. Photoperiod was controlled and adjusted to mimic the natural photoperiod throughout the acclimation and experimental periods.

Exposure system

The temperature of Hamilton city tap water closely follows the natural thermal regime of the inshore region of Lake Ontario, from which it is collected. Passage through the reverse osmosis unit unavoidably added 2°C to the water temperature; therefore, the ambient regime was consistently 2°C above the natural regime. Hereafter, the ambient regime will be referred to as the control temperature. After softening and deionization by reverse osmosis, the product water was pumped to a main head tank where hard water was added in a ratio of 1:40 to achieve $[Ca^{2+}]$ and $[Na^+]$ of ~50 and 100 μequiv·L^{-1}, respectively. This synthetic soft water (pH 6.3) was then gravity fed into two subhead tanks. The gravity feed to one of these two subhead tanks passed through a heat exchanger where 2°C was added to the control temperature. The water from each of these two tanks was further split into two more subhead tanks, resulting in four treatment head tanks. H_2SO_4 (0.2 M) was metered into one each of the control and +2°C temperature head tanks, resulting in the following four treatments: control temperature – control pH 6.3 (0/6.3); control temperature – sublethal pH 5.2 (0/5.2); control temperature + 2°C – control pH 6.3 (+2/6.3); and control temperature + 2°C – sublethal pH 5.2 (+2/5.2).

The water from the treatment head tanks flowed into duplicate 211-L polypropylene exposure tanks at an average rate of 1.2 L·min^{-1}, which allowed for 90% replacement in 7 h. Sublethal pH 5.2 was maintained by monitoring the pH in the treatment tanks using an industrial pH electrode (Leeds & Northrup Meredian II Combination). The electrode reading controlled a solenoid valve (Cole Parmer Instrument Co., CP No. 01367–70) that opened and closed according to the tank pH and the high and low pH control points, adding H_2SO_4 to the treatment head tank. Temperature and low pH were monitored via a programmable controller (Ladder Logic and Texas PLC model TI315), which was connected to an alarm and an automatic message dialer system (Safe House model 49-433A). The alarm system was triggered by a drop or increase in temperature of 1.0°C and (or) pH of 0.8 pH units. All tanks and head tanks were aerated, and water partial pressure of O_2 (P_{O_2}) was determined once a week using a thermostatted radiometer – P_{O_2} electrode (Copenhagen E5046) connected to an oxygen meter (Cameron Instrument Co.). P_{O_2}s averaged 115 Torr (1 Torr = 133.322 Pa) and did not drop below 80 Torr. Temperature and pH were measured daily. Ambient pH averaged 6.27 ± 0.03, and low pH averaged 5.27 ± 0.03. $[Na^+]$ and $[Ca^{2+}]$ were monitored twice a week using atomic absorption spectroscopy (Varian AA-1275) and

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averaged 97.5 ± 2.23 and 57.7 ± 2.28 μequiv·L^{-1}, respectively. Titratable alkalinity was determined once a week by titration to an end point of pH 4.2, using 0.02 M HCl. Mean values were 269.5 ± 18.8 and 120.3 ± 15.3 μequiv·L^{-1} for the control pH and low pH treatments, respectively. Water chloride (69.9 ± 14.5 μequiv·L^{-1}; spectrophotometric determination; Zall et al. 1956) and total chlorine (10–12 μg·L^{-1}; Hach, Loveland, Colo.) were measured every 30 days.

Feeding regime

At the end of the 8 weeks of acclimation, 142 trout were selected at random and placed in each of the eight treatment tanks, with 152 fish being placed in one of the 0/6.3 treatment tanks to allow 10 extra fish for day 0 sampling. Fish were hand-fed Zeigler's Trout Starter No. 3 to satiation twice daily at 08:30 and 16:30. The food was changed to Trout Starter No. 4 (of identical composition) halfway through the exposure because of fish growth. An aliquot of food from preweighed bags was sprinkled on the water surface of each replicate tank. If all of the food was consumed within 1 min, more food was placed on the water surface. When food was left on the surface at the end of the 1-min period, a second minute was allowed. If food remained after this 2-min period, the fish were assumed to be satiated and feeding was stopped. If not, the process was repeated until feeding stopped. At the end of each day, the bag was reweighed to determine the amount of food consumed. Fish consumed ~10–11% of their dry body weight·day^{-1} of dry food on this feeding regime. Fecal material was removed from the tanks by siphon every day, and tanks were scrubbed clean once a week.

Physiological measurements

Sampling protocol

Sampling and physiological measurements were conducted over a 4-day period every 30 days, including the first day of the experiment. The tests done on each of the 4 days are outlined below. Trout were not fed on the 3rd and 4th days of the sampling period, when whole-body and blood samples were collected. Methods were modified for nitrogenous waste excretion and oxygen consumption measurements on days 30, 60, and 90 relative to day 0. These changes were primarily dictated by the need for longer periods of aeration between sampling because of the higher summer temperatures present at these times.

Nitrogenous waste excretion

Day 0 (experiment initiation): In-tank routine nitrogenous waste excretion was measured over a 24-h period on the 1st day of each 30-day exposure period by stopping water flow to the tank. Aeration ensured mixing, and tests demonstrated no loss of ammonia as a result of aeration. Initial and final water samples were taken at time 0 and 1 h. The flow to the tank was then resumed for 1 h, and the sampling and closure continued at times 3 h and 4 h. This cycle was continued over the 24-h period. Samples were frozen at –20°C for later analysis of ammonia by the salicylate–hypochlorite assay (Verdouw et al. 1978) and of urea by the diacetyl monoxime method (Rahmatullah and Boyd 1980), as modified by T.P. Mommsen (see Lauff and Wood 1996). For the latter, the samples were first freeze-concentrated 5-fold by lyophilization. Nitrogen production (N; total ammonia and urea nitrogen) was determined by the difference between N concentration at the beginning and end of each closed-tank period, factored by time, volume, and fish weight.

Days 30, 60, and 90: Nitrogenous waste excretion was measured on a flow-through basis on the 1st day of each 30-day exposure period by taking an inflow and outflow water sample for each treatment every 4 h over a 24-h period. Inflow water rate was measured for each sampling period. Samples were frozen and later analyzed for urea and ammonia nitrogen as above. The difference between inflow and outflow total N concentration, factored by flow rate and fish weight, gave the nitrogen production rate in μmol·g^{-1}·h^{-1}. For each of the six sampling periods, data were graphed against time and the area under the curve was determined for each treatment to give an overall daily nitrogen production rate.

Fractional protein utilization (FPU) was determined for each treatment at each sampling period. FPU is an index of the fraction of Mo_2 that supports protein metabolism. It is calculated by dividing the nitrogen quotient (NQ = the ratio of moles of nitrogen produced to moles of oxygen consumed) by the theoretical maximum NQ in which protein supports all aerobic metabolism. This theoretical maximum for fish was determined by Kutty (1972) to be 0.27. In effect, the FPU represents the degree to which the fish depend on protein as an aerobic fuel source.

Metabolic rates

Day 0 (experiment initiation): Metabolic rates were determined on the 2nd day of the exposure period by measuring in-tank routine oxygen consumption rates (Mo_2) every other hour (alternating open and closed periods) over a 10-h period from 07:00 to 17:00. Fecal matter was first siphoned from the tanks. Aeration and inflow water were removed, the tanks were then sealed with a clear, air-tight lid, and water was recirculated within each tank using a submersible pump (Little Giant, 1 EUAA-MD). A water sample was removed from mid-tank every 20 min using a 5-mL syringe, and this sample was injected into a jacketed radiometer – Po_2 electrode (Copenhagen E5046) with ambient temperature water circulating through the jacket. The Po_2 was recorded on an oxygen meter (Cameron Instrument Co.) and was never allowed to decrease below 80 Torr. Mean Mo_2 values, determined for each hour using oxygen solubility coefficients from Boutilier et al. (1984), were graphed against time. The curve produced was integrated, for each treatment, to give an overall mean Mo_2 value for that 10-h period.

Days 30, 60, and 90: The same methods as above were used on the 2nd day of each 30-day exposure period, except that the O_2 electrode was suspended directly in the tank rather than in an external thermostatted cuvette.

All nitrogen excretion and Mo_2 data were corrected for fish size differences using the weight exponent 0.824, determined for rainbow trout by Cho (1992).

General procedures for blood and tissue analysis

Blood analysis

Ten fish were randomly chosen from each duplicate treatment tank (10 fish were taken from the control tank (0/6.3) only for day 0 sampling). Each fish was quickly killed by a blow to the head and blotted dry, and weight and total length were determined. Blood was collected from the dorsal aorta by caudal severance into ammonium heparinized capillary tubes. The blood samples were spun in an IEC MB micro hematocrit centrifuge (Damon/IEC Division) for 5 min at 10 000 × *g*. Hematocrit was determined as the ratio of the length of packed red blood cells to the total sample length and multiplied by 100 to give a percentage value. Because of the small size of the fish, it was important to conserve blood; the plasma in the capillary tube was removed, using a 50-μL Hamilton syringe, and frozen for subsequent ion analysis. Plasma Na^+ and Ca^{2+} levels were determined by atomic absorption spectroscopy, and Cl^- levels were determined by coulometric titration (Radiometer CMT10). When volume permitted, plasma protein was measured using a hand-held refractometer (American Optical) (Alexander and Ingram 1980).

Whole body and muscle samples

Ten fish were selected, without conscious choice, from each duplicate treatment tank and placed in terminal anaesthetic (1.0 g·L^{-1} MS-222 and 2.0 g·L^{-1} $NaHCO_3$). Each fish was blotted dry, weight and total length were determined, and the fish was rapidly frozen using aluminum tongs frozen in liquid N_2. Fish were wrapped individually in

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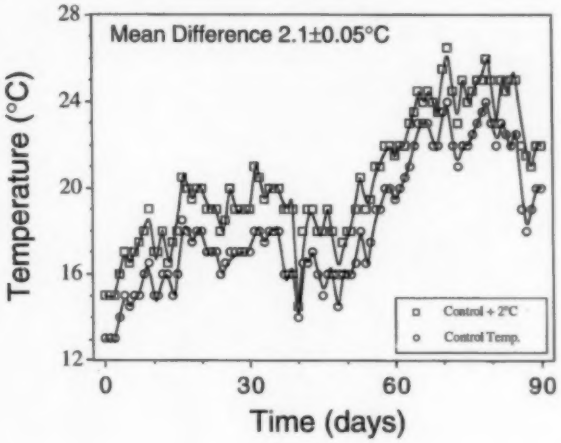
Fig. 1. Thermal cycle (June 18 – September 15, 1993) experienced by juvenile rainbow trout over the 90-day experimental period. Control temperatures peaked between days 60 and 90 at 24°C (26°C in the +2°C treatments).



labelled aluminum foil and stored at –80°C for later whole-body analysis. Whole fish were ground frozen, using an IKA (M10/M20) grinding mill cooled to approximately –72°C. A sample of the tissue was weighed and then dried to constant weight at 80°C to determine water content. The remainder of the tissue was lyophilized (Labconco Lyph-Lock 6), desiccated, and stored at –20°C. Whole-body Na^+, Ca^{2+}, K^+, and Cl^- levels were determined as above for plasma ions after 100 mg of tissue was digested for 48 h in 900 µL of 1 M H_2SO_4 at 80°C. Subsamples of freeze-dried tissue were used to determine whole-body protein levels, using the Lowry assay as modified by Miller (1959). Lipids were extracted and quantified using the chloroform–methanol (2:1) method (Folch et al. 1957). Glycogen, glucose, and lactate levels were determined as an estimate of whole-body carbohydrate, using standard enzymatic analyses (Bergmeyer 1985). Percentage inorganic content (ash) was determined by burning a subsample of whole-body tissue at 550°C in a muffle furnace until a constant weight was achieved.

A portion of the white muscle was removed from the dorsal section posterior to the operculum, and anterior to the dorsal fin, from the fish used for blood sampling above. The skin and fin rays were removed, and the white muscle was frozen in liquid nitrogen, wrapped in labelled aluminum foil, and stored at –80°C for later analysis. The samples were weighed and then dried at 80°C to a constant weight to determine water content.

Mortalities

Few mortalities occurred as a result of the treatments over the 90-day period; they amounted to less than 10% overall. Unfortunately, because of a laboratory mishap, the +2/5.2 treatment duplicate tank was lost at day 23.

Energy budget

Because we determined the quantity of food consumed by trout in each treatment, the weight gain, the proximate components of that weight gain (protein, lipid, and carbohydrate), metabolic expenditure, and excretory losses, we have calculated a crude energy budget on an individual fish basis for each treatment over the 90-day period. It is crude in that these measurements were made on whole tanks of fish rather than on individuals, although corrected to an individual fish basis. However, the energy budget allows for examination of each of the inputs and outputs of energy in common units (Soofiani and Hawkins 1985). The transformation of each of the inputs and outputs into energy units (kilojoules) allows for a more accurate assessment of growth and energy conversion as it relates to energy gain because each of the components of new tissue (protein, lipid, carbohydrate) have different energy values. The energy budget, therefore, should show the effects of treatments on the allocation of energy in the present study and give a composite measure of the cost of living for trout in each treatment.

The basic energy budget equation is $C = P + M + U + F$, where C equals the total energy intake, P is the total energy gain, M is metabolic expenditure, U is the excretory nitrogen energy loss, and F is fecal loss. It is important to note that there are other energy losses for which we are unable to account, such as energy lost in the excretion of mucus sloughed from the surface of the body and the production of mucus tubes that envelop the feces (Shehadeh and Gordon 1969). Because fecal losses were estimated by difference, these unaccountable energy losses are included in the estimated value for fecal energy losses. We refer to F, then, as fecal and unaccounted energy losses. The sum of the energy gains, expenditures, and losses should equal the energy consumed. If the energy source is not adequate to meet the expenses of daily living, then endogenous stores will be mobilized and the fish should have a negative P value. To determine the energy equivalences of the nutrient components of food and fish, conversion factors from Jobling (1994) were used.

Statistical analysis

Values are given as the mean ± SEM (n = number of fish). Individual data from the replicate tanks were combined in all analyses. Mean values were compared using one-way ANOVA, and in cases where the F value indicated significance, the Tukey–Kramer comparison of all pairs test was applied to determine treatment differences within a sampling period. The accepted probability level for significance was $p < 0.05$.

Results

Experimental temperature regime

The control temperature ranged from 13 to 24°C, and the +2°C treatments ranged from 15 to 26°C (Fig. 1). Control temperature rose from 13 to 16–18°C over the first 20 days, after which it was fairly stable until day 55. Thereafter, the temperature rose to a maximum of 24°C at day 70 and fluctuated around this temperature until day 82. At this point, the temperature started to decrease and reached 20°C on day 90.

Consumption, growth, and condition

From day 0 (experiment initiation), the appetites of the trout in all treatments averaged ~10% body weight·day^{-1} (dry food/dry weight) until days 60–90. At ~day 70, appetites started to fall, and trout consumed about 5% body weight·day^{-1}. Overall, the trout exposed to control temperatures tended to consume more than the trout exposed to +2°C, and those exposed to low pH tended to eat more than their respective controls (Fig. 2A). Appetites began to differ at ~day 45, with the fish treated with control temperatures tending to consume more than the fish in the +2°C treatments. By day 90, it was apparent that the trout exposed to sublethal low pH were consuming more than their respective controls.

At day 90, growth was greatest in the control temperature treatments (Fig. 2B). The 0/5.2 fish grew significantly more than either of the +2°C treatments but not more than 0/6.3. The +2/6.3 treatment trout grew significantly less than the 0/6.3 trout.

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Fig. 2. (A) Cumulative appetites, expressed on a dry weight of food basis (grams per fish), and (B) absolute growth (grams), expressed as wet body mass, over each 30-day period. Significant differences are indicated by treatment groups that do not share a common letter.

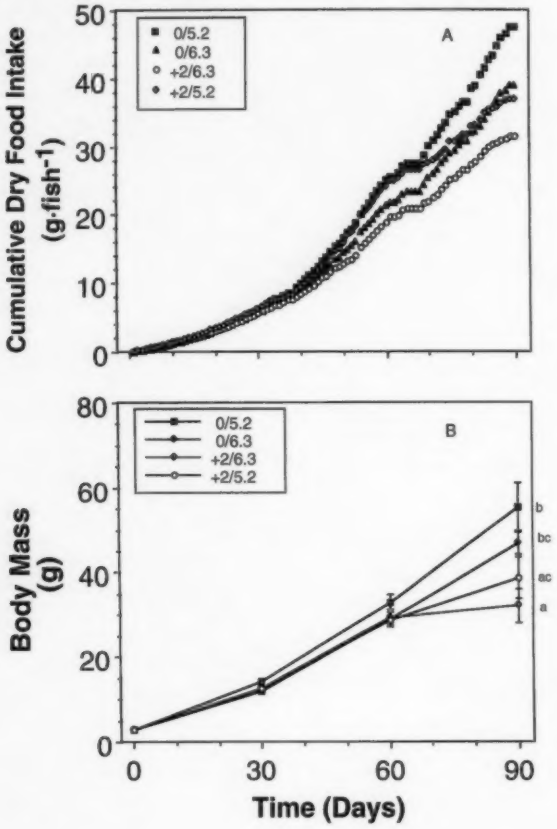


Table 1. Food conversion efficiencies for each 30-day period.

	Treatment			
Time period (days)	0/6.3	0/5.2	+2/6.3	+2/5.2
0–30	42.4	46.6	45.6	40.3
30–60	29.5	27.2	33.8	24.5
60–90	27.3	28.3	6.2	20.9

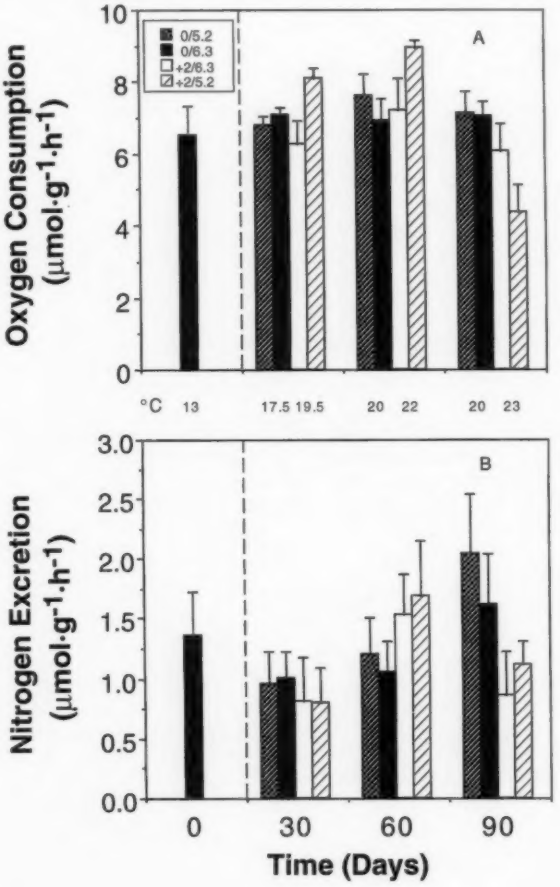
Note: Food conversion efficiencies calculated as ratio of dry weight of food to dry weight of fish (%·day^{-1}). Treatment regimes are described in the Materials and methods.

The condition factor (weight/length^3; Bagenal and Tesch 1978) of the trout in all treatments increased over the 90-day exposure period from ~0.9 to 1.2, although the efficiency with which dry weight of food was converted to dry weight of tissue decreased over the 90 days from ~40 to 25% (Table 1). At day 90, conversion efficiencies were lowest in both +2°C treatments. We have no explanation for the unusually low conversion efficiency in the +2/6.3 treatment between days 60 and 90.

Metabolic and nitrogenous waste excretion rates

Oxygen consumption measurements indicated that routine

Fig. 3. (A) Routine in-tank oxygen consumption ($\dot{M}o_2$) and (B) nitrogen excretion (total ammonia + urea nitrogen) rates (μmol·g^{-1}·h^{-1}). Rates were determined for the total number of fish in one duplicate tank, over a 10- and 24-h period, respectively, during the day, including feeding periods. Numbers between the graphs are the temperatures at which the rates were determined. The broken line separates day 0 (initial values) from the other measurements because satiation feeding did not begin until day 0. Errors bars represent measurement standard error only, and thus no statistical comparisons can be carried out.



values for these satiation-fed fish were about 7.0 μmol·g^{-1}·h^{-1} (Fig. 3A). Notably, there was a lack of increase in $\dot{M}o_2$ with increasing temperatures over the summer exposure. Among treatments, there was a trend towards a higher $\dot{M}o_2$ in the +2/5.2 treatment until day 60 and day 90 $\dot{M}o_2$ values were depressed.

Nitrogenous waste excretion rates decreased initially; thereafter they steadily increased to about 1.75 μmol·g^{-1}·h^{-1} at day 90 (Fig. 3B). About 10–20% of this nitrogenous excretion was represented by urea nitrogen. Over the 90-day period, this fraction decreased from ~20 to 10% as a result of increased ammonia nitrogen excretion and relatively constant urea nitrogen excretion. At days 60 and 90, the fish in the low pH treatments

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Table 2. Fractional protein utilization (FPU) in four treatment regimes.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
FPU	0.78	0.52	0.52	0.48	0.37	0.59	0.56	0.78	0.70	1.07	0.85	0.52	0.96

Note: FPU is calculated as (moles N produced/moles O_2 consumed)/0.27, where the denominator is the theoretical maximum nitrogen quotient as determined by Kutty (1972). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

Table 3. Measured hematocrit and plasma protein for each treatment at 30-day periods.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
Hematocrit (%)	46.2	33.1	35.5	33.1	32.8	39.0	38.8	41.2	38.7	33.1	33.2	31.8	30.9
±SEM	1.5	1.2	1.4	2.1	1.2	0.8	1.1	0.9	1.2	1.9	1.4	0.7	1.1
n	20	20	19	19	10	20	17	18	10	9	12	11	6
Plasma protein ($g \cdot 100\ mL^{-1}$)	5.6	5.6	5.3	5.7	5.5	5.6	5.2	5.5	5.6	5.3*b*	4.6*ab*	4.4*a*	3.8*a*
± SEM	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.2	0.3	0.1	0.2	0.3
n	10	10	13	10	5	10	10	12	8	9	12	11	6

Note: Mean values ± SEM and sample size (*n*) are shown. Significant differences between treatments occurred for plasma protein at day 90 only, and at this time, treatments that share a letter are not significantly different from each other ($p < 0.05$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

tended to excrete more nitrogen than their respective controls, and excretion rates were depressed in the +2°C treatments at day 90.

Fractional protein utilization values indicate that protein use generally increased in all treatments over the 90-day period. At day 90, protein use was lowest in the +2/6.3 treatment (Table 2).

Blood analysis

Initial values of hematocrit on day 0 were high at 46% (Table 3), perhaps reflecting the difficulty that we experienced in obtaining blood from such small fish (3 g). On days 30, 60, and 90, hematocrits were between 35 and 40% in all treatment groups, and no treatment differences were evident. Plasma protein levels remained unchanged at ~5.5 $g \cdot 100\ mL^{-1}$ until day 90 (Table 3). At this point, plasma protein was significantly higher in the 0/5.2 treatment than in the two +2°C treatments, although none was different from the 0/6.3 treatment.

Plasma Na^+ generally increased over time in all treatment groups from the initial value of ~105 to 150 $mequiv \cdot L^{-1}$ (Fig. 4A). Between days 60 and 90, the +2/5.2 treatment had significantly lower levels of plasma Na^+ than both control temperature treatments. Plasma Cl^- did not mirror this trend (Fig. 4B). Because plasma was limited at day 0, there is no initial value for plasma Ca^{2+}. At subsequent sampling times, plasma Ca^{2+} was ~4.5 $mequiv \cdot L^{-1}$ with no apparent influence of treatment (data not shown).

Proximate analysis

Whole-body analysis indicated that the percentage of protein by weight (wet) generally increased from ~12.0 to 15.5% over the 90-day period (Fig. 5A). Protein content was significantly greater in all treatments than in the control (0/6.3) at day 30. At day 60, protein content was significantly greater in the control temperature treatments than in the +2°C treatments. However, neither of these trends was maintained at day 90.

There was a substantial increase (~2.5-fold) in whole-body lipid content over the 90-day period from ~4.5 to 11.5% in all treatments (Fig. 5B). Among treatments, lipid content was significantly greater in the 0/5.2 treatment and lower in the +2/6.3 treatment than in the 0/6.3 treatment, at day 60 only.

Total carbohydrate (estimated as glucose + glycogen + lactate) did not show any consistent trend among treatments or over time and constituted only ~0.36% of whole-body composition (data not shown). Percentage whole-body water decreased over time in all of the treatments from 77 to 71% (data not shown), compensating for the large increase in lipid. There was no change in total inorganic content (ash; data not shown).

Whole-body Na^+, Cl^-, Ca^{2+}, and K^+ levels indicated that treatment effects were minor (Table 4). $[Na^+]$ and $[Cl^-]$ tended to decrease over the 90-day period, $[Ca^{2+}]$ increased, and $[K^+]$ was not affected. At day 90, the 0/5.2 treatment fish had significantly lower whole-body $[Cl^-]$ than the control treatment, and whole-body $[Ca^{2+}]$ was significantly elevated in the +2/5.2 fish.

Energy budget

A greater total amount of energy was consumed by the fish in the 0/5.2 treatment than in all other treatments (Table 5). That food energy was also converted more efficiently into energy gained. Metabolic expenditure was similar in all treatments, although slightly more energy was lost, in the form of nitrogen, by fish in the 0/5.2 and +2/5.2 treatments. Fecal and unaccounted energy losses were greatest in the +2/6.3 treatment fish and generally greater in the two +2°C treatments than in the control temperature treatments.

Discussion

The present study is the first to assess the effects of chronic sublethal low pH and an elevated summer thermal cycle on freshwater fish. Our original hypothesis was that the cost of living in a polluted environment, such as anthropogenically acidified soft water, would be further increased by the addition

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Fig. 4. (A) Plasma Na^+ and (B) Cl^- levels (mequiv·L^{-1}). Values are given as means ± SEM. Significant differences ($p < 0.05$) are indicated by treatment groups that do not share a common letter.

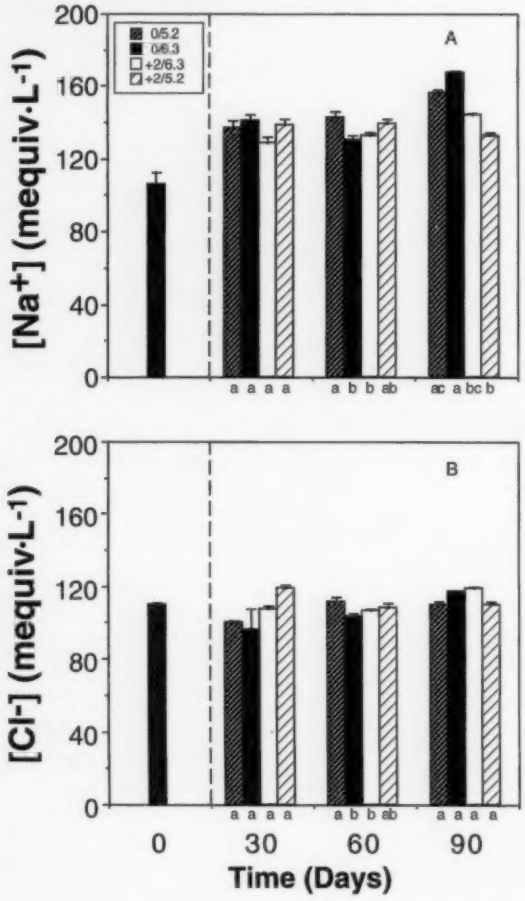
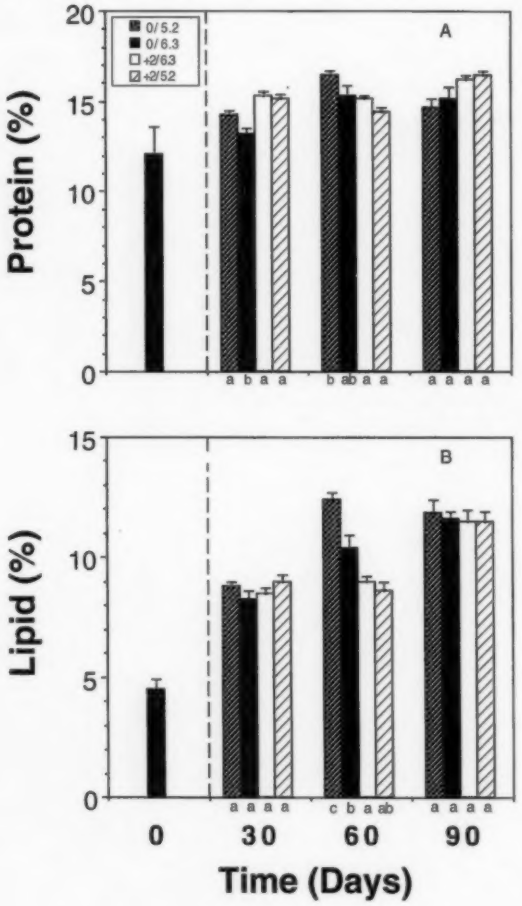


Fig. 5. (A) Whole-body protein and (B) lipid (%, wet weight). Values are given as means ± SEM. Significant differences ($p < 0.05$) are indicated by treatment groups that do not share a common letter.



of 2°C to the natural thermal cycle. As a result of the high pressure required for reverse osmosis, an additional 2°C was added to the ambient thermal cycle, resulting in an overall increase in temperature of 4°C. It is also one of the few studies (Kwain 1975; Menendez 1976; Leino and McCormick 1992; Wilson et al. 1994*a*) to examine the effects of these environmental stressors on fed fish. Often, studies investigating environmental stressors, such as pollutants, are carried out on starved or weight-maintained fish (Neville 1985; Audet et al. 1988; Booth et al. 1988; Butler et al. 1992). In the wild, and particularly in the summer months, fish actively feed as a consequence of both increased temperatures and food availability.

Temperature effects

Metabolic rate is a measure of the integrated cost of whole-body physiological and biochemical processes. In fish, it is affected by temperature, fish size, and food intake and assimilation (the heat increment of feeding (Cho and Kaushik 1990), also known as apparent specific dynamic action (SDA) (Beamish 1974*b*)). After correcting for weight increases, the most striking aspect of the routine $\dot{M}O_2$ data in the present study was that they were about 75% of $\dot{M}O_2$(max) values (Wilson et al. 1994*b*; also corrected for weight). This general effect was likely due to the feeding regime used. The increased cost of metabolism as a result of feeding is well documented (Beamish 1974*b*; Brett 1976; Soofiani and Hawkins 1982; Cho and Kaushik 1990). Ration size and composition and temperature determine the extent of the increase in oxygen consumption (Cho et al. 1982). Soofiani and Hawkins (1982) found that for cod fed to satiation, $\dot{M}O_2$ almost equalled the active metabolic rate. This dramatic increase in $\dot{M}O_2$ has important consequences for the scope for activity (difference between routine and active metabolic rates; Brett 1956) in fish. The authors found that this effect reduced the scope for activity in cod (*Gadus morhua* L.) by 83–97%, depending on the temperature. However, Brett (1976) found that the increase in $\dot{M}O_2$ as a result of feeding had less of an impact on the scope for activity in fingerling sockeye salmon (*Oncorhynchus nerka*).

The rates of most physiological processes increase with

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Table 4. Whole-body [Na^+], [Cl^-], [Ca^{2+}], and [K^+] (mequiv·kg^{-1}) for each treatment at each 30-day period.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
n	10	19	20	18	10	20	18	19	10	12	18	12	6
[Na^+]	45.4	43.6	46.5	42.2	44.3	37.5	37.8	39.7	38.5	36.2*a*	40.9*ab*	48.7*b*	37.7*ab*
±SEM	0.4	2.8	4.0	0.7	0.6	1.1	0.9	0.6	1.1	2.0	0.7	5.9	1.1
[Cl^-]	40.1	35.8	38.7	33.8	35.7	30.2	29.7	30.2	29.6	26.5*b*	31.9*a*	33.7*a*	27.9*ab*
±SEM	0.6	1.8	3.4	0.6	0.4	0.9	0.6	0.5	1.0	2.0	0.6	1.1	1.1
[Ca^{2+}]	73.3	85.2	84.9	76.8	77.3	84.6	86.1	88.7	87.5	81.4*a*	84.6*ab*	79.3*a*	92.0*b*
±SEM	1.4	4.7	6.9	1.0	1.9	1.7	1.2	1.1	2.1	1.9	1.3	2.6	1.9
[K^+]	83.2	86.6	105.1	92.9	87.1	87.4	93.5	88.9	87.1	84.9*a*	85.8*a*	84.5*a*	84.6*a*
±SEM	2.3	4.3	9.6	1.3	1.7	2.3	2.0	1.7	3.0	1.8	1.2	1.7	2.9

Note: Mean values ± SEM and sample size (*n*) are shown. Significant differences occurred between treatments at day 90 only. During this period, treatments that share a letter are not significantly different from each other ($p < 0.05$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

Table 5. Energy budget for each treatment for the 90-day period.

	Total energy consumed (*C*)		Total energy gained (*P*), kJ	Conversion efficiency (*K*), %	Total metabolic expenditure (*M*)		Total N energy lost (*U*)		Fecal and unaccounted energy lost (*F*)	
Treatment	kJ	%			kJ	%	kJ	%	kJ	%
0/5.2	963.9	100	480.6	49.9	288.7	30.0	51.8	5.4	142.9	14.8
0/6.3	837.1	100	369.7	44.2	249.5	29.8	38.3	4.6	179.6	21.5
+2/6.3	785.8	100	254.4	32.4	218.9	27.9	29.0	3.7	283.5	36.1
+2/5.2	723.0	100	283.6	39.2	224.0	31.0	34.5	4.8	180.9	25.0

Note: The general energy budget equation is $C = P + M + U + F$, where *C* is the energy consumed, *P* is the energy gained, *M* is metabolic expenditure, *U* is excretory energy loss, and *F* is fecal and unaccounted energy loss. Fecal and unaccounted losses are estimated by the difference ($C - (P + M + U)$). Each component of the budget is expressed as a percentage of *C* to determine allocation differences. *K* is the efficiency with which food energy is converted into total energy gain ($C/P \times 100$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

increasing temperature (Schmidt-Nielsen 1987), the rate increase often expressed as a Q_{10}. For example, Mehner and Wieser (1994) found that $\dot{M}O_2$ values in juvenile perch (*Perca fluviatilis*) acclimated to 20°C had a Q_{10} of 2.43 when compared with perch acclimated to 15°C. Claireux et al. (1995) found that resting cod, acclimated to 5°C, exhibited a Q_{10} of 2 in $\dot{M}O_2$ when the temperature was increased to 7.5°C. Calculations based on the data of Fig. 3A indicated that Q_{10} values averaged only ~1.0 in the present study. Fish acclimate more rapidly to increases in temperature than to decreases in temperature (Jobling 1994). Because the temperatures gradually increased over the 90-day test period, the fluctuating and increasing thermal regime involved in the present study may have allowed for continuous acclimation, thereby permitting complete metabolic compensation and thus no apparent increase in metabolic rates. Thermal history, therefore, is an important parameter to be considered when determining metabolic expenditures, especially when predictions of growth are important.

Temperatures approaching the upper lethal temperature for salmonids have been shown to depress appetite and metabolic rates (Brett 1971; Elliott 1982) and, subsequently, growth (Brett et al. 1969). The upper incipient lethal temperature for rainbow trout (26.2°C; Elliott 1982) was reached in the +2°C treatments. However, the increasing temperatures over the previous 60 days allowed for gradual acclimation and may well have increased thermal tolerance in that no mortalities occurred over the 10-day peak temperature period. The depression in appetite and growth in the +2°C treatments, notably at day 90, and the decline in gross conversion efficiency in the +2°C treatment over the period of days 60–90 indicate that 2°C added to the control thermal cycle significantly affected trout, particularly after peak temperatures were reached. A 20% reduction in protein turnover was also evident in these fish during this period of peak temperatures (Reid et al. 1995) and is further evidence of the suppressive effects of high temperature on metabolic processes.

Endogenous energy catabolism did not increase in these fish, as indicated by stable whole-body protein, lipid, and carbohydrate levels, even though food intake was reduced. The suppressive effects of high temperature on appetite and metabolic rates discussed above may be extended to endogenous energy catabolism in an overall effort to conserve energy by reducing the energy expended on SDA. Although this could be considered a pathological response, it may also be an adaptive strategy used by the fish in the face of high temperature conditions.

Addition of 2°C tended to increase nitrogen excretion rates, until the peak in temperature was reached. At day 90, nitrogen excretion rates in the +2°C treatments were reduced. Because endogenous nitrogen use did not increase, as indicated by the maintenance of whole-body protein levels, this reduction was due to the suppression of appetite during the 60- to 90-day period. A reduction in exogenous protein intake, and no reliance on endogenous protein sources, is further supported by the reduction in use of protein, as indicated by the low fractional protein utilization (FPU) value in the +2/6.3 treatment.

In general, addition of 2°C to the naturally fluctuating control

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thermal cycle appeared beneficial to rainbow trout until their optimal temperature for growth was surpassed. This temperature has been determined by Cho and Kaushik (1990) to be between 15 and 20°C. Control temperatures, for the first 50 days, fluctuated between 15 and 18°C, and a separation in appetites was evident at day 50, just as the temperature began its incline to the peak temperature at day 70. It was over this period that the inhibitory effects of the additional 2°C on appetite and growth became most evident.

Low pH effects

The greater appetites and better growth exhibited by trout exposed to sublethal acidity than their respective controls were contrasted with those of many previous studies showing deleterious effects of acidic pH. For example, some investigators have reported that sublethal low pH increases $\dot{M}O_2$ (Hargis 1976; Waiwood and Beamish 1978; Butler et al. 1992), indicating increased metabolic cost, and reduces growth in fish. Menendez (1976) and Sadler and Lynam (1987) found that growth in brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) was impaired at pH levels below 5.2. The important difference in the present study was that trout were fed to satiation. In this regard, Wilson and Wood (1992) reported no difference in growth for rainbow trout fed 1% body weight·day^{-1} and concluded that chronic exposure to pH 5.2 did not deleteriously affect the energy budget of fed fish. Indeed, in a separate study, Wilson et al. (1994*a*) reported increased growth in trout that were exposed to pH 5.2 and fed to satiation for 35 days, a result similar to that of the present study. Conversion efficiencies of acid-exposed trout were not, in general, different from the control, indicating that the subsequent increased growth was purely a result of increased appetite. It is noteworthy, then, that chronic exposure to pH 5.2 should apparently stimulate appetite. This was also evident in the +2/5.2 treatment discussed below, although growth was not significantly different from control growth.

Proximate analysis indicated that increased appetites in the 0/5.2 treatment resulted in deposition of more protein and lipid at days 30 and 60, respectively. This trend was not maintained at day 90, as a result of the peak in temperature over the period of days 60–90. Also, a tendency for higher nitrogen excretion rates denoted increased protein catabolism (as a result of increased food intake rates). It is clear from the above discussion that chronic exposure to low pH in trout does not reduce growth when trout are simultaneously fed to satiation in the laboratory environment.

Combination treatment effects

Trout exposed to both low pH and increased temperature generally expended more metabolic energy. The greater depression in $\dot{M}O_2$ in the +2/5.2 treatment than in the +2/6.3 treatment, after the day 70 peak in temperature, indicated greater physiological impact of the peak temperatures on the former. These trout had appetites greater than those of the fish exposed to +2°C alone and equal to those of the 0/6.3 treatment. However, they did not exhibit better growth than the trout in the +2/6.3 treatment. This result implies that the increased cost of living in this +2/5.2 environment was being paid in the form of greater immediate use of the exogenous energy, resulting in less energy available for deposition as growth. This is supported by the lower conversion efficiencies exhibited by these fish at all periods.

The amount of energy taken in was sufficient to maintain endogenous energy stores as indicated by the similar protein and fat deposition in all treatments to the control. After the period of peak temperature, it is evident that the trout in the +2/5.2 treatment were reducing both food intake and $\dot{M}O_2$. Consequently, nitrogen excretion rates were reduced, arguing that endogenous sources were not used. Energy conservation was clearly of paramount importance during this period.

Indicators of stress

Exposure to chronic sublethal low pH did not result in decreases in plasma Na^+ and Cl^- concentrations (cf. Audet et al. 1988), nor did it cause fluid shifts, increased hematocrit, or increased plasma protein concentrations. There was, however, a reduction in whole-body chloride in the 0/5.2 treatment relative to its control at day 90 only. The general absence of ionoregulatory disturbance in this experiment contrasts with other chronic studies, such as that carried out by Leino and McCormick (1992) on juvenile largemouth bass, where mean blood osmolalities declined at pH 5.0 and 4.5. Audet et al. (1988) showed conclusively that the main toxic effect of chronic exposure to sublethal low pH (pH 4.8) in adult rainbow trout was ionoregulatory disturbance, manifested by a partial inhibition of Na^+ and Cl^- influx and a reduction of Na^+ and Cl^- efflux. In their study, plasma Na^+ and Cl^- concentrations were reduced but stabilized at a lower level, while plasma protein concentration was increased. However, the fish in their study were fed only once per week. Wilson et al. (1994*a*) demonstrated that juvenile rainbow trout, exposed to pH 5.2 for 35 days, recovered whole-body [Na^+] and [Cl^-] to control levels, after a nadir at day 17. This recovery was attributed to "enough time"; these fish were fed to satiation twice a day, a feeding regime duplicated in the present study.

As pointed out by Smith et al. (1989), very little research has been directed at accounting for ions taken in as part of the diet and the role that they play in ionoregulation. We have calculated the dietary ion budget for our fish on the basis of food ion concentrations and whole-body ion pools. Trout in the low pH treatments were consuming, on average, 10% of their body pool of Na^+ per day in a food ration amounting to 10% of the dry body weight per day. If indeed there was an initial loss of ions, as described by Wilson et al. (1994*a*), the availability of ions in the food in combination with enough time might have allowed the observed recovery in their study and would account for the observed lack of ionoregulatory disturbance in the trout exposed to low pH in the present study. Sadler and Lynam (1987) found similar effects in yearling brown trout that were exposed to low pH conditions (4.4–5.2) and fed 2% of their body weight per day. In brown trout that were starved, however, reductions in plasma chloride concentration, increased muscle water content, and losses of body sodium and potassium were significant. The authors suggest that the fed trout may be making use of dietary ions to ameliorate the effects of low pH.

Energy budget

The data in Table 5 support the preceding discussion of greater appetites and thus weight gain in the 0/5.2 treatment. Energy intake (C) was greater in both control temperature treatments

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than in their respective +2°C treatments. The value beside each gross energy value indicates the percentage of the total energy intake allocated to each component of the energy budget. It is clear that the fish in the 0/5.2 treatment not only took in more energy, but also allotted a greater percentage of that energy intake to growth (energy gain; 49.9%). There were almost equal metabolic energy expenditures over the 90-day period, and the 0/5.2 trout lost more energy in the form of nitrogen than those in the other treatments, particularly the +2/6.3 treatment. However, lower estimated fecal and unaccounted energy losses (14.8%) indicated that not only were the 0/5.2 trout taking in a greater gross amount of energy, but they were also converting that energy more efficiently into gross energy gain (K). In contrast, and in agreement with the previous discussion, the +2/6.3 trout showed the least energy gain and the least energy expenditure, but the greatest fecal and unaccounted energy losses. It is also apparent from the data that the fish in the +2/6.3 treatment had a greater gross energy intake over the 90-day period than those in the +2/5.2 treatment. This is explained by a slightly greater absolute food intake over the period of days 60–90 than those in the +2/5.2 treatment, although appetites at all other periods were less than those in the +2/5.2 treatment fish. Nonetheless, the trout in the +2/6.3 treatment converted food less efficiently into energy gain than in all other treatments. In general, the fish in the two control temperature treatments consumed more food, gained more energy as growth, and converted the energy taken in into energy gain more efficiently than in the +2°C treatments. Also, fish in both low pH treatments exhibited better conversion efficiency and greater overall energy gain than in their respective controls.

Concluding remarks

The results of this 90-day exposure of juvenile rainbow trout to sublethal low pH, +2°C, and a combination of these two treatments indicate that, averaged over the entire exposure period, those trout exposed to +2°C exhibited a greater cost of living than those exposed to the control thermal regime. This is supported by the decreased food intake rates of trout exposed to higher temperatures, lower conversion efficiencies, and subsequent depressions in growth (energy gain). Metabolic rates were especially costly for these treatments over the period of days 60–90 when temperatures peaked, close to the incipient lethal temperature. It is evident, then, that a global temperature increase of 2°C, as a consequence of "greenhouse gas" production, may be deleterious to temperate cold-water fish species, particularly when temperatures reach the summer high.

Exposure of juvenile rainbow trout to sublethal levels of acidity resulted in greater food intake and growth rates (energy gain), notably at control temperatures. This may be an indication that sublethal levels of this particular toxicant stimulate increased energy intake to compensate for increased costs associated with living in an acidic environment. These higher costs could be a consequence of ionoregulatory disturbance. If so, appetite might be driven by the requirement for NaCl through the diet. This stimulus, coupled with an unlimited food supply, may result in an overcompensation for the increased cost of living in an acidic environment in the form of increased growth. Smith et al. (1989) stated that under conditions of impairment of branchial sodium uptake, such as under acidic conditions, dietary ions may play a critical role in body ion homeostasis. Sadler and Lynam (1987) indicated that compensation for increased energy expenditure and ionoregulatory imbalance, as a result of moderately low levels of pH, may be occurring when fish are given sufficient food. It is uncertain how much food juvenile salmonids consume in the wild, but an unlimited ration is not realistic. It is also uncertain how much energy a fish expends searching for food and avoiding predators. However, it is unlikely that the unlimited food supply available to the trout in the present study is representative of food availability in the wild, because $\dot{M}O_2$ values were at 75% of $\dot{M}O_2$(max) as a consequence, we propose, of SDA. We suggest that future studies on global warming – low pH scenarios should examine possible effects of food limitation. Limiting food may elucidate whether there are actually increased costs associated with living in a sublethally stressful environment and what role food intake rates play in compensation.

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Phylogeographic origins of lake trout (*Salvelinus namaycush*) in eastern North America

Chris C. Wilson and Paul D.N. Hebert

Abstract: There is little consensus regarding either the number or relative importance of Pleistocene glacial refugia occupied by lake trout (*Salvelinus namaycush*). Mitochondrial DNA restriction fragment length polymorphisms were analyzed to test existing hypotheses concerning the postglacial origins and dispersal of *Salvelinus namaycush* in eastern North America. Analysis of fish from geographically widespread populations with 20 restriction enzymes revealed three distinct mitochondrial lineages. These lineages last shared a common ancestor approximately 0.1 to 0.7 million years ago, during the middle to late Pleistocene. Screening of 862 fish from 60 populations with five diagnostic enzymes showed that the different lineages had largely allopatric distributions, suggesting their dispersal from separate refugia. Populations in New England, the Maritimes, and most of Quebec were established by fish from an Atlantic refugium, which also contributed to populations in Ontario. Ontario populations were dominated by Mississippian colonists, although populations in northwestern Ontario and Quebec also contained Beringian lake trout. Secondary contact was extensive among refugial groups, with 22 of the 60 populations containing fish from more than one refuge. These results demonstrate that both contributions of non-Mississippian refuge populations and admixture among lake trout refugial groups are much greater than previously thought.

Résumé : On note peu de consensus sur le nombre ou l'importance relative des refuges glaciaires du Pléistocène occupés par le touladi (*Salvelinus namaycush*). L'analyse du polymorphisme de longueur des fragments de restriction de l'ADN mitochondrial a servi à vérifier les hypothèses formulées au sujet de l'origine post-glaciaire et de la dispersion de *Salvelinus namaycush* dans l'est de l'Amérique du Nord. L'analyse de les poissons appartenant à des populations géographiquement dispersées présentant 20 enzymes de restriction a montré trois lignées mitochondriales distinctes. L'ancêtre commun de ces lignées remonte à environ 0,1 à 0,7 million d'années, dans la période du Pléistocène moyen à tardif. Le criblage de 862 poissons appartenant à 60 populations à l'aide de cinq enzymes diagnostiques a montré que les différentes lignées présentaient des distributions généralement allopatriques, ce qui permet de penser qu'elles se sont dispersées à partir de refuges distincts. Les populations de Nouvelle-Angleterre, des Maritimes et de la plus grande partie du Québec ont été établies par des poissons provenant d'un refuge de l'Atlantique, qui a aussi contribué à la constitution de certaines populations de l'Ontario. Les populations de l'Ontario étaient dominées par des pionniers mississipiens, mais certaines populations du nord-ouest de l'Ontario et du Québec contenaient aussi des touladis béringiens. Les contacts secondaires étaient fréquents entre les groupes provenant des divers refuges, puisque 22 des 60 populations contenaient des poissons provenant de plus d'un refuge. Ces résultats démontrent que les contributions des populations provenant des refuges autres que le mississipien, et le mélange entre les groupes de touladis des divers refuges, sont beaucoup plus importants qu'on ne le pensait jusqu'ici.
[Traduit par la Rédaction]

Introduction

Despite a half century of debate, the postglacial origins and dispersal of the lake trout (*Salvelinus namaycush*) have yet to be resolved. Its natural distribution closely matches the North American limits of the Wisconsinan ice sheets (Lindsey 1964), where it is limited to lakes carved by glacial scouring. Fossil evidence suggests that lake trout were able to closely follow the retreating glacial margins, as the species was present in northern Wisconsin (Hussakof 1916) 12 500 – 16 000 years ago (Lindsey 1964) and in eastern Ontario approximately 10 000 years ago (Gruchy 1968, in Crossman and McAllister 1986). Despite comprehensive reviews regarding the species' Pleistocene refugia and postglacial dispersal (Martin and Olver 1980; Crossman and McAllister 1986), the number and locations of lake trout glacial refugia are still unresolved. On the basis of distributional patterns, workers have argued for the species' dispersal from a single Beringian (Radforth 1944), single Mississippian (Walters 1955), or multiple refugia (Lindsey 1964). Early morphological investigations (Khan and Qadri 1971) supported the view that lake trout persisted in at least four refugia (Atlantic, Mississippian, Missourian, and Beringian), but subsequent multivariate tests (Crossman and McAllister 1986) limited the likely refugia to two (Mississippian and Beringian). Data on the parasitic nematode *Cystidicola stigmatura* suggested multiple lake trout refugia, with eastern populations of *Salvelinus namaycush* originating from Mississippian and Atlantic sources (Black 1983). Data interpretation was complicated, however, by the dependence of the parasite on a secondary host (*Mysis relicta*) that has limited dispersal abilities (Dadswell 1974). Allozyme work (Ihssen et al. 1988) detected two groups of lake trout in Ontario and Manitoba, which were interpreted as Mississippian and Atlantic in origin, as well as a third minor group (Haliburton area,

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Fig. 1. Map of *Salvelinus namaycush* sampling sites in eastern North America. Names of sampled populations are provided in Table 1.

Ontario) that was inferred to have originated from a third refuge. Using analysis of mitochondrial DNA (mtDNA) diversity, Grewe and Hebert (1988) discovered three distinct lineages with differing geographic distributions among Ontario hatchery stocks, which were described as Atlantic, Mississippian, and Beringian.

Mitochondrial DNA has emerged as a powerful tool for examining biogeographic events, both above and below the species level (Wilson et al. 1985; Avise et al. 1987; Moritz et al. 1987). Its maternal inheritance, lack of recombination, high mutation rate relative to nuclear DNA, and selective neutrality make mtDNA an ideal tool for studying historical populations and dispersal events (Wilson et al. 1985; Avise et al. 1987). This approach has already been used to evaluate the history and dispersal of several North American freshwater fish species (Bermingham and Avise 1986; Billington and Hebert 1988; Ward et al. 1989; Bernatchez and Dodson 1990, 1991; Murdoch and Hebert 1996) and has shown great potential for resolving the intraspecific phylogeny of *Salvelinus namaycush* on the basis of analysis of hatchery populations (Grewe and Hebert 1988).

We analyzed mtDNA diversity to assess the phylogeography and postglacial dispersal of lake trout in eastern North America, as one component of a project examining the species' origins and dispersal across its entire range. Our results confirm the origins of eastern populations from multiple refugia and indicate that secondary contact between refugial groups is much more extensive than previously thought.

Methods

Eight hundred and forty-two lake trout were collected from 59 native populations in eastern Canada and the United States between 1990 and 1994 using gill nets, trap nets, and creel surveys (Table 1, Fig. 1). Sample sizes ranged from 3 to 36 fish per population, with a mean value of 14.5. Wherever possible, lake trout were obtained either from or close to populations used in earlier studies (Lindsey 1964; Khan and Qadri 1971; Black 1983). To provide Beringian fish for comparison, 20 lake trout from Toolik Lake in unglaciated northern Alaska (Flint 1971) were also analyzed. In most cases, fish were killed and their livers removed for mtDNA extraction. Livers were stored in 30 mL of TEK buffer solution (Chapman and Powers 1984) with the addition of 0.25M sucrose (TEKS), and kept on ice or at 4°C until processed (1–12 days). For some remote or vulnerable populations, fish were biopsied by removing adipose fin clips, which were preserved in 95% ethanol.

Mitochondrial DNA was extracted from livers using Chapman and Powers' (1984) protocol, as modified by Danzmann et al. (1991). Liver tissue was homogenized in 30 mL of TEKS buffer using a hand-held grinder at low speed on ice. The homogenate was then

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Table 1. Native lake trout populations sampled in eastern North America, showing lake numbers for Fig. 1, map coordinates, sample sizes, number of haplotypes present, and estimated nucleon (h) and nucleotide (π) diversities.

No.	Province or state	Lake	Latitude	Longitude	N	N_h	h	π
1	Labrador	Trouser	56°32′	61°46′	14	1	0	0
2	New Brunswick	Chamcook[a]	45°09′	67°06′	15	1	0	0
3	Maine	Hurd Pond	45°48′	69°00′	16	1	0	0
4		Long Pond[a]	45°58′	70°09′	5	1	0	0
5		Moosehead[a]	45°40′	69°38′	15	2	0.133	0.015
6		Togue Pond	46°56′	68°53′	11	1	0	0
7		West Carry Pond	45°10′	70°07′	10	1	0	0
8	New Hampshire	Winnepesaukee	43°36′	71°22′	12	1	0	0
9	New York	Raquette	43°48′	74°36′	15	1	0	0
10		Seneca	42°45′	76°55′	7	2	0.571	0.129
11	Quebec	31 Milles	46°12′	75°49′	23	2	0.474	0.107
12		Albanel	51°05′	73°00′	12	3	0.318	0.111
13		Archambault	46°19′	74°15′	11	1	0	0
14		Argiles	45°52′	75°34′	12	2	0.303	0.067
15		Baie Comeau	49°32′	68°30′	18	1	0	0
16		Beland[a]	48°51′	73°19′	12	1	0	0
17		Boucher	49°30′	68°59′	20	1	0	0
18		Chasseurs	48°13′	67°52′	7	1	0	0
19		Gagnon	46°07′	75°07′	7	1	0	0
20		Joinville	46°18′	75°12′	10	1	0	0
21		Kuujuak R.	62°09′	78°10′	23	2	0.237	0.053
22		Lahaie[b]	48°28′	78°43′	5	1	0	0
23		Letemplier	49°27′	68°47′	20	1	0	0
24		Normand[a]	46°29′	73°14′	24	1	0	0
25		Mistassini	50°30′	73°30′	23	4	0.625	0.173
26		Polonais	47°00′	75°22′	7	1	0	0
27		Sault[b]	48°30′	78°42′	9	1	0	0
28		Simon	45°58′	75°05′	12	2	0.522	0.118
29		Sourd	46°09′	75°16′	11	1	0	0
30		Waconichi	50°07′	73°30′	24	2	0.356	0.080
31	Ontario	Buchanan	48°05′	86°00′	11	1	0	0
32		Burchell	48°35′	90°38′	5	1	0	0
33		Charleston	44°32′	76°00′	11	2	0.509	0.115
34		Clean–Clear	45°15′	78°32′	30	1	0	0
35		Como	47°55′	83°30′	5	2	0.600	0.135
36		Confederation	51°05′	92°44′	10	2	0.200	0.023
37		Daniels[a]	49°55′	93°50′	14	2	0.143	0.096
38		Elliot	46°23′	82°42′	11	2	0.510	0.115
39		Flack	46°35′	82°47′	9	3	0.417	0.113
40		Gordon	49°54′	93°43′	14	1	0	0
41		Hawley	54°30′	84°39′	9	1	0	0
42		Joseph	45°10′	79°44′	34	2	0.166	0.037
43		Lavieille	45°51′	78°14′	3	1	0	0
44		Louisa	45°28′	78°29′	15	1	0	0
45		Little Sparkling	49°50′	90°13′	12	1	0	0
46		MacDonald	45°14′	78°34′	24	1	0	0
47		Mameigweiss	49°34′	91°49′	17	2	0.221	0.050
48		Niobe	48°43′	91°20′	14	1	0	0
49		Nipigon[a]	49°50′	88°30′	30	7	0.782	0.337
50		Opeongo[a]	45°42′	78°23′	30	2	0.129	0.029
51		Paguchi	49°34′	91°32′	12	1	0	0
52		Radisson	48°13′	80°45′	3	1	0	0
53		Scattergood[a]	49°18′	92°43′	10	3	0.711	0.277
54		Slate Island[c]	48°40′	87°00′	36	4	0.519	0.176
55		Smoke	45°31′	78°41′	6	1	0	0
56		Superior[a]	48°00′	88°00′	21	7	0.832	0.347
57		Tim[a]	45°45′	79°02′	15	1	0	0

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Table 1 (*concluded*).

No.	Province or state	Lake	Latitude	Longitude	N	N_h	h	π
58		Vista	49°05′	93°05′	4	1	0	0
59	Wisconsin	Trout	46°02′	89°49′	17	1	0	0
60	Alaska	Toolik[a]	68°38′	149°36′	20	1	0	0

Note: Toolik Lake (Alaska) was included for comparison. Nucleotide diversity (π) values have been multiplied by 100.
[a]Populations used for surveying mtDNA heterogeneity for 20 restriction enzymes.
[b]Data provided by Neil Billington, University of Southern Illinois at Carbondale.
[c]Samples from first-generation hatchery population taken from spawning adults, Slate Island, Lake Superior.

Table 2. Restriction enzymes used to examine *Salvelinus namaycush* mtDNA and their resulting fragment patterns.

Enzyme	Fragment patterns
	Monomorphic enzymes
*Apa*I	7.90, 3.88, 1.75, 1.41, 1.31, 0.66
*Bcl*I	8.26, 7.41, 1.07
*Dra*I	7.05, 5.35, 3.30
*Eco*RI	8.80, 8.00
*Hae*II	8.34, 3.84, 1.75, 1.69, 1.04
*Kpn*I	No cut sites
*Mlu*I	No cut sites
*Nsi*I	11.14, 2.35, 1.58
*Pst*I	12.61, 3.97
*Pvu*II	8.60, 6.12, 1.42, 0.90
*Xba*I	8.11, 5.35, 3.18
	Polymorphic enzymes
*Ase*I[a]	
A	3.02, 2.93, 2.75, 2.19, 2.00, 1.75, 1.75, 0.57
B	3.02, 2.75, 2.19, 2.15, 2.00, 1.75, 1.75, 0.80, 0.57
C	2.93, 2.75, 2.19, 2.10, 2.00, 1.75, 1.75, 1.49
*Ava*I[a]	
A	5.49, 2.68, 2.22, 2.12, 1.68, 1.36, 1.10, 0.57
B	5.49, 2.68, 2.22, 1.68, 1.52, 1.36, 1.10, 0.60, 0.57
C	5.49, 2.68, 1.84, 1.68, 1.52, 1.36, 1.10, 0.60, 0.57, 0.37
D	5.49, 2.68, 1.84, 1.68, 1.36, 1.10, 0.90, 0.62, 0.60, 0.57, 0.37
*Bam*HI[a]	
A	16.80
B	9.48, 7.32
C	9.48, 5.00, 2.32
*Bst*EII	
A	9.46, 7.17
B	9.46, 6.55, 0.68
*Eco*O109I[a]	
A	4.39, 3.88, 2.75, 1.17, 0.73, 0.67, 0.64, 0.40, 0.30
B	4.39, 3.15, 2.75, 1.17, 0.84, 0.73, 0.67, 0.64, 0.40, 0.30
C	4.71, 4.39, 2.75, 1.06, 0.67, 0.64, 0.40, 0.30
*Hin*dIII[a]	
A	9.10, 2.24, 1.96, 1.76, 1.48, 0.26
B	9.10, 3.44, 2.24, 1.76, 0.26
C	9.10, 2.24, 1.96, 1.76, 1.74
*Nco*I	
A	7.46, 6.83, 1.83, 1.19
B	8.29, 7.46, 1.83

Note: Letter designations for polymorphic enzymes indicate separate fragment patterns.
[a]Enzymes used for geographic survey of lake trout populations.

subjected to repeated centrifugation at low speeds to remove nuclei and cellular debris. Mitochondria were subsequently pelleted using high-speed centrifugation, then resuspended in 2–3 mL of TEKS and lysed with 2% P-40 nonionic detergent. The lysed membranes were removed by centrifugation in a microcentrifuge at 4°C, and the mtDNA was purified via organic (phenol–chloroform) extraction. The purified mtDNA was precipitated using 100% ethanol, then dried and resuspended in 100–300 μL of 10 mM TE buffer. For adipose fin samples, both nuclear and mitochondrial DNA was extracted using a CTAB extraction protocol (Saghai-Maroof et al. 1984).

Mitochondrial DNA samples were digested with restriction enzymes and run on 0.8–1.2% agarose gels alongside molecular size standards (lambda phage DNA digested with *Hin*dIII, and double digested with *Hin*dIII and *Eco*RI). Gels were stained with ethidium bromide (EtBr), visualized on an ultraviolet transilluminator, and photographed for later reference. Sizes of the digested mtDNA fragments were calculated by comparison against the two size standards. Fragment patterns (haplotypes) for enzymes used previously (Grewe and Hebert 1988) were identified by their earlier designations. Fragment patterns generated by other enzymes were assigned letter designations in order of their detection. Fragment patterns generated by the different restriction enzymes for individuals were pooled to produce composite haplotypes.

If samples were of poor quality or were difficult to interpret after EtBr visualization, the DNA was denatured, neutralized, and transferred to a nylon membrane (Amersham Hybond-N) by vacuum or capillary blotting. Membranes were baked at 80°C for 2 h, stored, and visualized by probing with purified lake trout mtDNA labeled with a chemiluminescent chemical (digoxigenin (DIG), Boehringer Mannheim). Membrane hybridization and autoradiography were carried out as described by Höltke et al. (1992).

Forty-eight fish from 12 geographically distant populations including Toolik Lake were screened for variation with 20 restriction endonucleases (Table 2), including several enzymes known to provide useful phylogenetic information in lake trout (Grewe and Hebert 1988). Restriction fragment homologies were confirmed using double digests with reference enzymes (*Eco*RI, *Dra*I). Estimates of pairwise nucleotide divergences among haplotypes were calculated using equations from Nei and Tajima (1981). Genetic relationships among haplotypes were determined using both character-based and phenetic analyses with the PHYLIP 3.5c computer package (Felsenstein 1993). Estimates of divergence times among mtDNA lineages from ancestral populations were calculated using the formulae of Wilson et al. (1985) and an estimated rate of 1% divergence per million years for the salmonid "mtDNA clock" (Smith 1992).

Fish from the remaining populations were subsequently screened with five polymorphic enzymes that discriminated among the major mtDNA lineages (Table 2). Genetic variation within populations and relatedness among co-occurring haplotypes were calculated with the REAP computer package (McElroy et al. 1992), using the nucleon (h) and nucleotide (π) diversity equations of Nei and Tajima (1981) and Nei (1987). Values for the latter measure (π) were calculated with the assumption that monomorphic restriction sites observed in the pilot survey (Table 2) were invariant among all populations. Although this

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Table 3. Composite haplotypes for restriction enzyme polymorphisms detected among lake trout from 12 representative populations, showing numbers and locations of fish with each haplotype.

Haplotype	Composite fragment pattern							*N*	Location
1	A	A	A	A	A	A	A	11	49, 50, 53, 56
2	A	A	A	B	A	A	A	1	49
3	A	A	A	A	C	A	A	2	36, 54
4	A	A	A	A	A	B	A	15	57
5	A	A	B	A	A	A	A	1	56
6	C	A	A	A	A	A	A	2	25
7	A	B	B	A	A	A	A	23	2, 4, 5, 16, 25, 49, 53, 56
8	A	B	B	A	A	C	A	1	39
9	A	B	C	A	A	A	A	1	5
10	B	B	B	A	A	A	A	1	49
11	A	C	C	A	B	A	A	6	56, 60
12	B	C	C	A	B	A	A	7	37, 53, 49, 56
13	A	D	C	A	B	A	A	3	49, 56
14	B	C	C	A	B	A	B	1	56
15	B	D	C	A	B	A	A	1	49

Note: Variable enzymes from Table 2 are listed in order (*Ase*I, *Ava*I,*Bam*HI, *Bst*EII, *Eco*O109I, *Hin*dIII, *Nco*I). Names for locations are given in Table 1.

assumption was probably incorrect, it prevented overinflation of estimated π values.

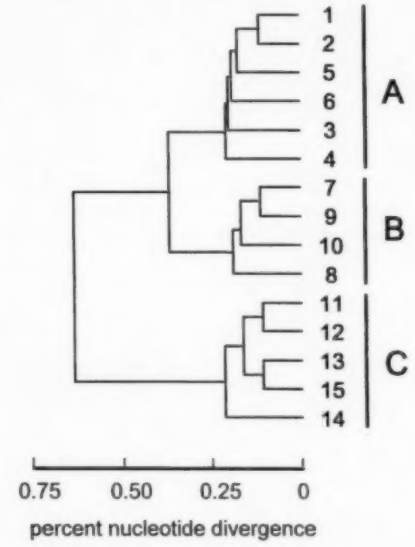
Results

Of the 17 hexameric and 3 multihexameric restriction enzymes used to screen fish from the 12 test populations (Table 2), 2 enzymes (*Kpn*I and *Mlu*I) failed to cut lake trout mtDNA. The remaining 18 enzymes recognized an average of 79 cut sites per fish, or 2.7% of the *Salvelinus namaycush* mtDNA genome. Restriction sites for 11 enzymes (38 cut sites) were conserved among the 12 populations, but the remaining 7 enzymes produced variable restriction patterns (Table 2), which revealed 15 haplotypes (Table 3).

Character-based analysis of the observed haplotypes was unable to resolve their phylogenetic relationships, owing to homoplastic recognition sites detected by *Ase*I and *Bam*HI (Table 3). The ambiguous restriction fragment patterns in Table 3 (*Ase*I B for haplotype 10, *Bam*HI B for haplotype 5, and *Bam*HI C for haplotype 9) were confirmed as homoplasies via additional restriction enzyme digests (Wilson 1995), including enzymes that were diagnostic for discriminating among known *Salvelinus namaycush* mtDNA lineages (Grewe and Hebert 1988).

Figure 2 shows relatedness among the observed haplotypes based on estimated sequence divergences, corrected for the homoplastic restriction sites. The observed lineages were labeled A, B, and C to correspond with the nomenclature of Grewe and Hebert (1988). Each lineage contained a dominant haplotype with mutational derivatives (Fig. 2). Estimated pairwise sequence divergences among all haplotypes ranged from 0.11 to 0.91%, with a mean value of 0.44%. The number of haplotypes within each group was relatively low, with group A having the greatest diversity. The estimated sequence divergence (mean ± 1 SE) between groups A and B was 0.37 ± 0.02%,

Fig. 2. UPGMA clustering of composite *Salvelinus namaycush* haplotypes based on estimated percent nucleotide divergence (Nei and Tajima 1981), showing relationships within and among major lineages. Group labels (A, B, C) correspond with phylogenetic lineages identified by Grewe and Hebert (1988).



whereas group C differed from A by 0.75 ± 0.12% and B by 0.46 ± 0.12% (Fig. 2).

Following the determination of phylogenetically useful restriction enzymes, mtDNA from the remaining fish was screened with five enzymes (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, and *Hin*dIII) that varied among groups A, B, and C, to determine if any geographic patterns existed among haplotypes. Screening of the remaining populations with these enzymes revealed 13 haplotypes that corresponded with groups A, B, and C (Table 4). As fish could not be definitively assigned to haplotypes in Table 3 using the reduced set of enzymes, geographic haplotypes in Table 4 were renumbered by lineage. No additional major phylogenetic lineages were detected, and groups A, B, and C were readily discriminated using *Ava*I. *Bam*HI was similarly diagnostic, although homoplasies were observed for two fish with unique haplotypes (Tables 3 and 4). In addition, *Eco*O109I digests clearly discriminated lake trout belonging to group C from those in groups A and B (Table 3). Group C was similarly distinguishable with *Ase*I, although one group B fish was homoplastic for this enzyme (Table 4). *Hin*dIII was largely invariant, although minor haplotypes within groups A and B were detected.

The three mtDNA lineages showed differing geographic distributions in eastern North America (Fig. 3). Group A was the most common lineage throughout Ontario and was the only group observed in Wisconsin. By contrast, group B dominated populations in Quebec, New Brunswick, and New England, while group C was largely limited to populations in western Ontario. In addition, lake trout from one population in eastern Quebec (Lac des Chasseurs, $n = 7$) contained mtDNA identical to that for landlocked arctic char (*Salvelinus alpinus*) from that region (Wilson et al. 1996) (Fig. 3).

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Table 4. Composite lake trout haplotypes detected with five polymorphic restriction enzymes (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, *Hin*dIII) in the geographic survey of 60 populations, showing numbers of individuals with each haplotype and locations of less abundant haplotypes.

Haplotype	Composite fragment pattern					*N*	Location
A1	A	A	A	A	A	392	
A2	A	A	A	C	A	2	36, 54
A3	A	A	A	A	B	15	57
A4	A	A	B	A	A	1	56
A5	C	A	A	A	A	2	25
B1	A	B	B	A	A	388	
B2	A	B	B	A	C	1	39
B3	A	B	C	A	A	1	5
B4	B	B	B	A	A	1	49
C1	B	C	C	B	A	19	12, 25, 37, 49, 53, 56
C2	A	C	C	B	A	22	56, 60
C3	A	D	C	B	A	3	49, 56
C4	B	D	C	B	A	1	49

Note: Names for locations are given in Table 1.

Despite clear geographic shifts in haplotype frequencies, contact and admixture among the mitochondrial lineages was extensive (Fig. 3). Group A was present in several Quebec populations and made up most of the Kuujuak River population in northern Quebec. Group B fish occurred in several Ontario populations as far west as the Manitoba border, as well as in both New York populations. Similarly, group C lake trout dispersed as far as western Quebec.

Nucleon diversities were typically low, and varied with lake size (Table 1). Very large lakes (Superior and Nipigon) showed the most variation, while most lakes contained only a single haplotype. In the majority of lakes with more than one haplotype present, increased diversity was due to sympatry of fish from more than one of groups A, B, and C, resulting in increased levels of nucleotide diversity (Table 1, Fig. 3). In several lakes, however, secondary haplotypes were mutational derivatives of the dominant regional lineage, resulting in low values of π (Table 3, Fig. 2).

Most mutational derivatives within the major lineages observed in the pilot study were detectable with the five restriction enzymes used for the geographic survey (Table 3). The broad survey showed that these derivative haplotypes had localized distributions and typically occurred as small proportions of single populations (Table 4). One notable exception was haplotype 4, which was present in all 15 fish collected from Tim Lake (population 57) in western Algonquin Park (Ontario).

Haplotype distributions were not a consequence of past stocking programs. Only 5 of the 59 lakes were known to have been stocked, and none showed evidence of non-native fish. For three of these lakes (Charleston Lake in Ontario and Seneca and Raquette lakes in New York), stocked fish were derived from native spawners. However, Moosehead Lake in Maine has been stocked with lake trout from Lake Superior (P. Johnson, Maine Department of Fish and Game, personal communication), but fish from this lake had mtDNA typical of unstocked lakes in the area (Fig. 3). Similarly, Trout Lake in Wisconsin has been stocked with Great Lakes strains (Lyons 1984), but only the common group A haplotype was detected.

Discussion

The low number of haplotypes observed in most lake trout populations suggests that they either arose from small numbers of founder individuals or have maintained small population sizes over time (Avise et al. 1984). This observation is concordant with the low levels of allozyme diversity observed in lake trout populations (Ihssen et al. 1988). As long-lived predators in low-productivity (oligotrophic) habitats (Martin and Olver 1980), lake trout population sizes have probably been modest in all but the largest lakes. The low level of haplotype diversity is unlikely to be an artifact of limited sample size, as large samples (≥30 fish) from several lakes also had low nucleon diversity values (Table 1). The low nucleon diversities of many populations provide further support for their native status, as many hatchery stocks of lake trout contain three or more haplotypes (Grewe and Hebert 1988). Similarly, introduced populations of lake trout in western Canada founded from eastern hatchery stocks show high diversity levels typical of their parent strains (C.C. Wilson, unpublished data). The only eastern populations with haplotype compositions similar to those of hatchery strains are lakes Superior and Nipigon, a finding that reflects their use as sources for hatchery strains.

Levels of nucleon diversity among eastern lake trout populations are similar to those observed in other northern freshwater species. Overall nucleon diversity of populations in Table 1 ($h_t = 0.557$) was comparable to values for lake whitefish, *Coregonus clupeaformis* ($h_t = 0.733$,0.523) (Bernatchez and Dodson 1990, 1991), and walleye, *Stizostedion vitreum* ($h_t = 0.550$) (Billington and Hebert 1988). These values were considerably less than those for warm-water species such as brown bullhead, *Ameiurus nebulosus* ($h_t = 0.923$) (Murdoch and Hebert 1996), and *Lepomis* species in the southeastern United States ($h_t = 0.674$–0.961) (Bermingham and Avise 1986).

The mtDNA analysis identified three distinct lineages among the eastern North American populations. The mitochondrial molecular clock for salmonids has been estimated at 1% divergence per million years (Smith 1992), suggesting that the three groups last shared a common ancestor 0.1–0.7 million years ago, during the mid- to late Pleistocene. It should be noted that divergence time estimates based on molecular data are inexact and should be treated with caution, particularly when the number of characters upon which such estimates are based is low. Nonetheless, the data suggest that divergence among the *Salvelinus namaycush* lineages preceded the last (Wisconsinan) glaciation. The limited divergence observed within each lineage (Fig. 2) and the low numbers and abundances of minor haplotypes (Table 3) suggest that these represent mutational derivatives of the common haplotypes, rather than fish from separate subrefugia.

The genetic divergence and geographic distributions of groups A, B, and C strongly suggest that they dispersed from separate refugia. Lake trout with group B haplotypes likely originated from an Atlantic refuge and colonized much of New England and the Atlantic provinces. Similarly, the distribution of fish with group A haplotypes suggests their origin from a Mississippian refuge. Within the C group, the correspondence

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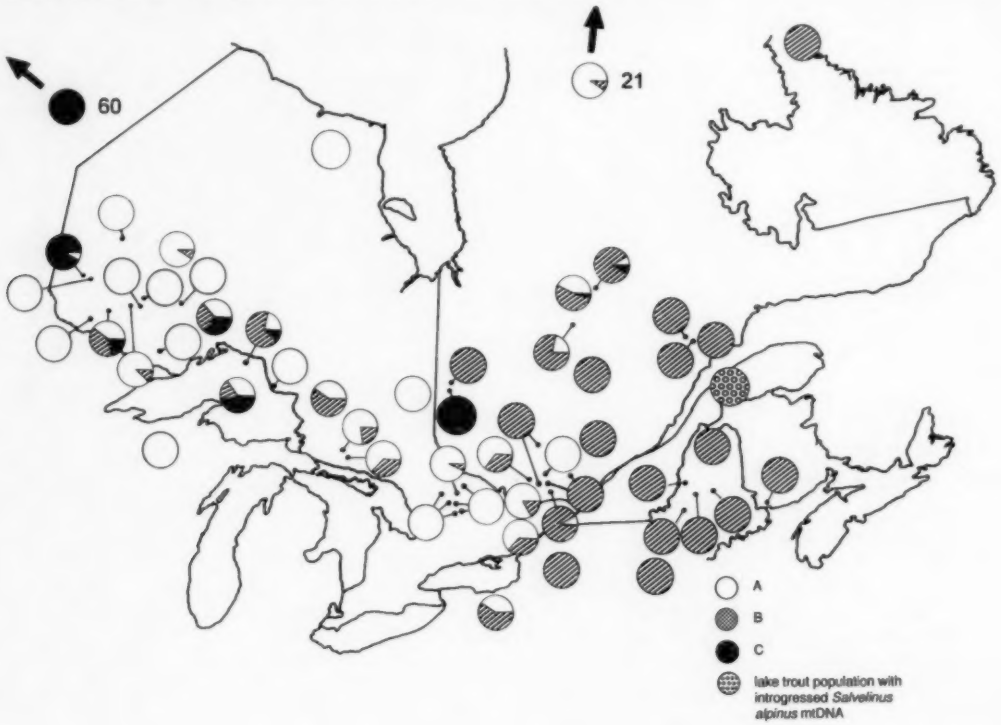
Fig. 3. Geographic distributions of major *Salvelinus namaycush* mtDNA lineages among populations in eastern North America. Numbers represent outlying populations: 21, Kuujuak River, Quebec; 60, Toolik Lake, Alaska.



of haplotypes in Ontario and Quebec populations with those in Toolik Lake fish suggests their Beringian origin. In addition, virtually all lake trout examined in a separate study from populations in the central and western Arctic had *Ava*I and *Bam*HI fragment patterns matching those for group C fish (Wilson and Hebert 1993), which strongly suggests that group C fish dispersed from a Beringian refuge.

Alternatively, it is possible that lake trout from more than one mitochondrial lineage were present in a given refuge. Thus, some haplotypes would not necessarily be diagnostic or indicative of dispersal from a particular refuge. Although it seems clear that the Atlantic refuge was occupied solely by group B lake trout, it is possible that group B and C haplotypes were present in the Mississippian refuge. In this case, the presence of haplotypes from more than one lineage would not necessarily indicate secondary contact of fishes from separate refugia, and postglacial contact among refugial groups would be considerably less than if each lineage emanated from a separate refugial source.

This latter hypothesis seems unlikely, however, as the probable small population sizes of lake trout in peripheral refugia during glacial intervals would have been equivalent to sustained bottlenecks, with considerable loss of genetic diversity. Although it is probable that glacial refugia would originally have contained fish with a diverse array of haplotypes, population size constraints would have caused the eventual loss of much of the initial diversity (Avise et al. 1984). This is supported by genetic data from North American and Eurasian populations of whitefish, which show much lower levels of diversity within North American populations, suggesting that fish populations in North American refugia were smaller or more severely affected (Bernatchez et al. 1989). This also seems to be indicated by the low diversity and limited divergence within each of the lake trout lineages observed. Thus, it seems reasonable to assume reciprocal monophyly for lake trout refugial groups, and the presence of a mtDNA lineage as resulting from postglacial dispersal via proglacial lake connections.

The mtDNA data refute earlier hypotheses of a single post-Pleistocene origin for lake trout (Radforth 1944; Walters 1955) and support multiple-origin hypotheses (Lindsey 1964; Khan and Qadri 1971). Crossman and McAllister's (1986) reinterpretation of Khan and Qadri's meristic data set is not supported by the mitochondrial data. Instead, the mtDNA results agree with studies suggesting that both Atlantic and Mississippian refugia played important roles as sources for eastern lake trout populations (Black 1983; Legendre and Legendre 1984; Black et al. 1986). These data also show that Radforth (1944) was partially correct in arguing that Beringian lake trout colonized eastern North America, a hypothesis discounted by all later workers. Indeed, Bailey and Smith (1981) found no evidence to support any fish species colonizing the Great Lakes from Beringia.

Among these previous studies, Black's (1983) use of parasitological data to infer lake trout dispersal from a Mississippian refuge showed the closest match to the distribution of

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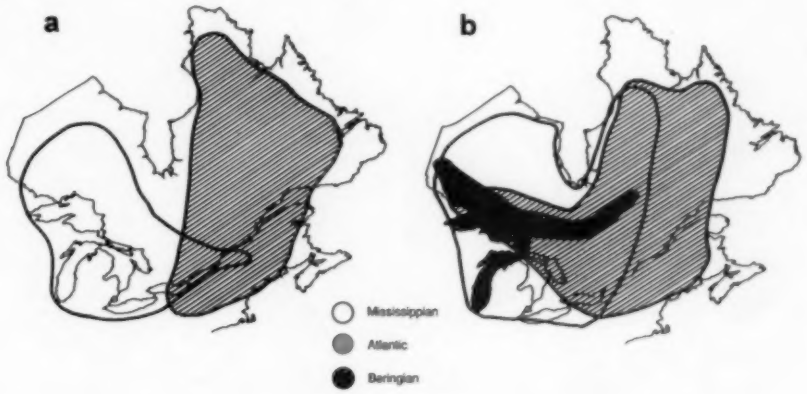
Fig. 4. Comparative predictions of refugial sources for eastern lake trout populations, based on (*a*) Black (1983), and (*b*) this study. Presence or absence of mtDNA groups in the lower Great Lakes in *b* is based on mtDNA data from hatchery and native stocks (Grewe and Hebert 1988; Burnham-Curtis 1993).



group A haplotypes of *Salvelinus namaycush*, although results from the two data sets differ in several important aspects (Fig. 4). As mentioned previously, the spread of the parasite marker was dependent on dispersal by its secondary host (*M. relicta*). Because of this, the absence of *Cystidicola stigmatura* from eastern lake trout populations could not demonstrate with certainty the existence or contributions of other refugia, although Black's (1983) data suggested an Atlantic refuge (Fig. 4*a*). In contrast, the mtDNA survey conclusively shows the contributions of both Atlantic and Beringian lake trout to recolonizing the study area (Fig. 4*b*). Finally, secondary contact among refugial groups as shown in Figs. 3 and 4*b* could not be assessed with the parasite data, because of the transmission of *C. stigmatura* throughout a population of mixed refugial origins once admixture occurred.

Exact locations of lake trout refugia remain uncertain, as changes in climate following glacial retreat have probably displaced lake trout from their Pleistocene sources (Martin and Olver 1980). Potential Atlantic refugia such as the Georges Bank or glacial Sable Island, for example, now form part of the submerged continental shelf (Schmidt 1986). Ice-dammed lakes in the Hudson River valley may also have sheltered Atlantic populations (Bailey and Smith 1981). Glacial Lake Wisconsin in the Driftless area (Wisconsin) was a probable Mississippian refuge, as it existed before and during the glacial maximum (Flint et al. 1959) and was proximal to the location of the oldest known lake trout fossil (Lindsey 1964). Lake trout populations also likely persisted in numerous temporary ice-dammed lakes along the glacial margins, subject to local extinction and dispersal (Bailey and Smith 1981).

Lake trout are capable of dispersing great distances: tagged individuals have moved hundreds of kilometres in a matter of weeks (Scott and Crossman 1973; Martin and Olver 1980). Their high vagility and widespread distribution suggest that they could have taken advantage of even transient connections among proglacial lakes and drainages (Mandrak and Crossman 1992). Indeed, the distributions of the lake trout mtDNA lineages observed in this study can readily be interpreted in light of connections among proglacial lakes during ice retreat (Fig. 5).

Lake trout from a Mississippian refuge were undoubtedly able to readily colonize Ontario, as they were present in precursors of the major proglacial lakes and encountered no dispersal barriers once the ice retreat began (Bailey and Smith 1981; Crossman and McAllister 1986). Group A fish had ready access to Lake Agassiz at least 14 000 years ago through the lake's Mississippi drainage outlet (Teller and Thorleifson 1983). As the Laurentide ice sheet retreated, the resultant large meltwater lakes south of the ice (Chigago, Warren, Duluth, Algonquin, Iroquois) also provided avenues for dispersal throughout the province (Fig. 5). Group A fish in northwestern Ontario could have arrived either through outflows from Lake Agassiz or via earlier stages of Lake Superior.

Lake trout dispersing from an Atlantic refuge also had access to the Great Lakes and Ontario (Fig. 5), through a connection between Lake Vermont (Belleville – Fort Ann phase) and Lake Iroquois approximately 12 000 – 11 000 years ago (Underhill 1986; Mandrak and Crossman 1992). Colonization of Quebec north of the St. Lawrence River probably occurred prior to the formation of the Champlain Sea (Underhill 1986), which appeared 11 000 years ago (Dyke and Prest 1987; Mandrak and Crossman 1992). This inland sea, which existed from 11 000 to 9800 years ago, would have formed a dispersal barrier for lake trout (Khan and Qadri 1971; Power et al. 1973) as well as other freshwater Atlantic species (Underhill 1986). Northern dispersal from New England became possible once again with the replacement of the Champlain Sea by Lampsilis Lake (9500–7800 years ago) (Mandrak and Crossman 1992).

Drainage from the upper Great Lakes (Glacial Lake Algonquin) through the Kirkfield and Fossmill outlets (12 000 – 11 000, 10 800 – 10 000 years ago) probably enabled colonization of eastern Ontario and western Quebec by Mississippian refuge fish (Underhill 1986; Mandrak and Crossman 1992). The virtual absence of group B fish from lakes near these drainage outlets (Fig. 3) suggests that Atlantic lake trout did not penetrate farther west before 10 800 years ago (Underhill 1986; Dyke and Prest 1987). Once in Quebec, lake trout were likely able to follow the retreating ice sheet and disperse throughout the province through a mosaic of headwater exchanges and lake interconnections (Legendre and Legendre

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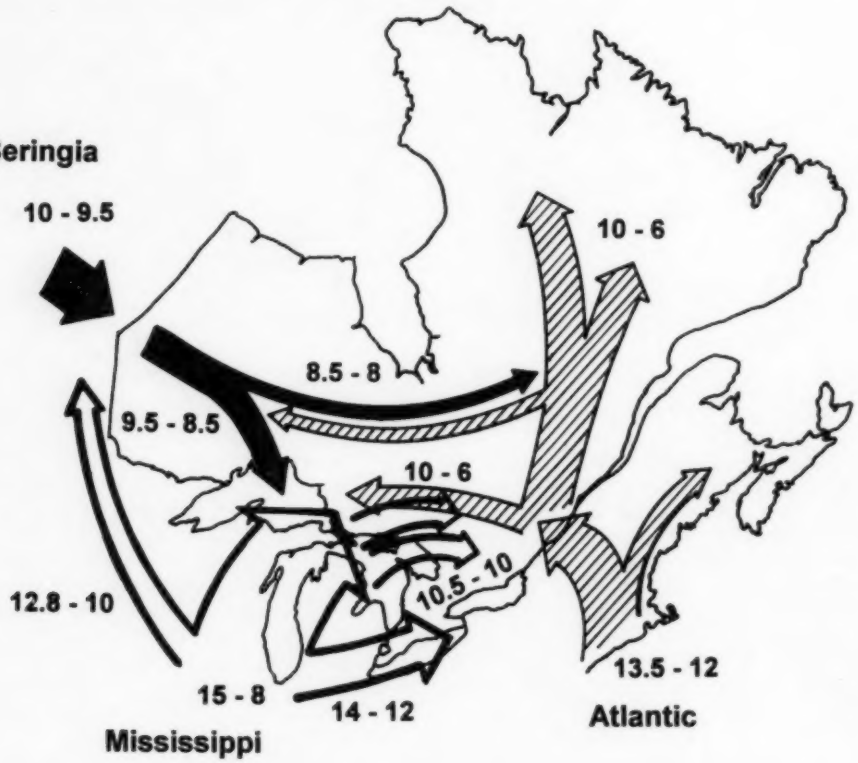
Fig. 5. Schematic representation of lake trout colonization of eastern North America from Beringian, Mississippian, and Atlantic refugia. Shading patterns for mtDNA groups match those in Fig. 4. Numbers represent probable timing of lake connections in thousands of years before present.



1984), as well as further overland dispersal into Labrador (Black et al. 1986). Atlantic fish in western Ontario probably arrived through entry into Lake Algonquin via the North Bay outlet (10 000 – 6000 years ago) (Mandrak and Crossman 1992).

Group C fish in eastern Canada emigrated from Beringia through direct connections between proglacial lakes Peace, McConnell, and Agassiz (Smith and Fisher 1993), arriving in Lake Agassiz during the Emerson phase 10 000 – 9600 years ago (Teller and Thorleifson 1983; Mandrak and Crossman 1992). Lake Agassiz was connected to the Great Lakes basin numerous times through the Nipigon spillway between 9500 and 8500 years ago (Teller and Thorleifson 1983), permitting colonization of eastern Canada by the Beringian lake trout. The high frequency of group C lake trout in Lake Michigan stocks (Grewe et al. 1993) indicates that Beringian colonists dispersed widely through the western Great Lakes.

Beringian lake trout probably colonized western Quebec from Lake Agassiz via a connection with Lake Ojibway–Barlow (Fig. 5), which covered significant portions of northeastern Ontario and western Quebec (Vincent and Hardy 1979). This dispersal route was short-lived, as the connection between lakes Agassiz and Ojibway–Barlow formed only 500 years before the abrupt disappearance of Lake Ojibway–Barlow with the formation of the Tyrrell Sea 8000 years ago (Vincent and Hardy 1979; Dyke and Prest 1987). A second fish species (goldeye, *Hiodon alisoides*) is thought to have colonized western Quebec from Lake Agassiz in a similar manner (McPhail and Lindsey 1970; Stewart and Lindsey 1983).

The mitochondrial data suggest that secondary contact among lake trout refugial groups has been much more extensive than previously recognized, with 22 of the 59 populations having mixed origins. Moreover, the true extent of secondary contact is likely to have been greater than that indicated by the mtDNA data, owing to stochastic losses of mitochondrial lineages either by bottlenecks (founder events or subsequent population collapses) or sustained small population size for many generations (Avise et al. 1984). The ubiquity of secondary contact probably did much to erode genetic divergence in the nuclear genome that may have developed during periods of isolation in separate refugia.

Colonization and dispersal largely ended approximately 6000 years ago, with the disappearance of the Laurentide ice sheet and its attendant proglacial lakes (Bailey and Smith 1981; Crossman and McAllister 1986; Underhill 1986; Mandrak and Crossman 1992). Distribution patterns of the different refugial groups were largely established, although headwater exchange still permits dispersal in much of Quebec today (Legendre and Legendre 1984).

Genetic and distributional data show that many freshwater

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species occupied both Atlantic and Mississippian refugia during the Pleistocene (Bailey and Smith 1981; Underhill 1986). Mitochondrial analyses of walleye (*Stizostedion vitreum*) populations show many parallels with lake trout, with evidence of *Stizostedion vitreum* dispersal from Atlantic, Mississippian, and western refugia, with distributions closely matching lake trout lineages (Billington and Hebert 1988; Billington et al. 1992). The largely concordant phylogeographic patterns between lake trout and walleye (Billington and Hebert 1988; Ward et al. 1989; Billington et al. 1992) show that these species responded similarly to zoogeographic events. Similarly, mtDNA from brook char (*Salvelinus fontinalis*), lake whitefish (*Coregonus clupeaformis*), and brown bullhead (*Ameiurus nebulosus*) also show significant contributions from Atlantic refugia, with the latter two species having more than one Atlantic origin (Bernatchez and Dodson 1991; Danzmann et al. 1991; Murdoch and Hebert 1996). Although lake whitefish show a broadly similar phylogeographic pattern to lake trout (Bernatchez and Dodson 1991), *Coregonus clupeaformis* in much of Quebec are derived from a Mississippian rather than Atlantic refuge, suggesting that the Champlain Sea formed a barrier to Atlantic fish. In addition, *Coregonus clupeaformis* lineages show less secondary contact and better correspondence with later proglacial lakes, suggesting that lake whitefish did not follow lake connections as closely. Similarly, allozyme evidence from yellow perch (*Perca flavescens*) showed that Atlantic refuge fish did not disperse farther west than Lake Ontario, with most populations originating from a Mississippian refuge (Todd and Hatcher 1993).

Comparison with other studies shows that the dispersal of aquatic biota in eastern North America varied considerably among species. Fish and invertebrate species with otherwise poor dispersal abilities were transported huge distances by proglacial lakes and occur today solely within former lake margins (Dadswell 1974), while several deepwater fish species show limited dispersal outside these limits (Dadswell 1972). Many species from Atlantic refugia dispersed only as far as the Lake Ontario basin (Bailey and Smith 1981; Underhill 1986; Bernatchez and Dodson 1991; Murdoch and Hebert 1996; Todd and Hatcher 1993), whereas lake trout and walleye were apparently able to follow retreating glacial margins closely and colonize more distant areas. Differing phylogeographic patterns among these species may also be due to different numbers of colonists and priority effects, as the probability of a haplotype being lost from a population is inversely proportional to its abundance and is strongly influenced by population size (Avise et al. 1984). Therefore, the size of resident populations may be important in determining the relative reproductive contribution of new arrivals. This could potentially produce the result that species with different carrying capacities could show qualitatively different phylogeographic patterns despite having similar ranges, habitats, and levels of dispersal. As top predators, lake trout and walleye may be expected to have smaller population sizes than species in lower trophic levels such as whitefish. If the effect of local population size on haplotype establishment is significant, then the observed phylogeographic differences among these species may be due to their ecology and not dispersal alone.

The discrepancy between lake trout and other species with respect to eastward colonization from their Mississippian refuge is puzzling. Although populations of several other fish species in Quebec, the Maritimes, and New England show evidence of being colonized from Mississippian sources (Underhill 1986; Bernatchez and Dodson 1991), eastward movement of group A lake trout appears to have been extremely limited. As dispersal opportunities were certainly available (Dyke and Prest 1987), it may be that for lake trout these areas were colonized first by Atlantic refuge fish, and later arrivals from the Mississippian refuge were excluded because of priority effects.

The biogeography of aquatic species in eastern North America shows both marked similarities and differences when compared with that for freshwater species examined by Bermingham and Avise (1986) and Avise (1992) in the southeastern United States. In both regions, habitat disturbance and dispersal pathways were primary factors influencing the genetic structure of resident species, resulting in broadly similar intraspecific phylogeographic patterns within each region. Qualitative differences in intraspecific phylogeographies are apparent between the two regions, however. Many southern species have intraspecific sequence divergences of 5–8% with high nucleon diversity (Bermingham and Avise 1986; Avise 1992). In contrast, northern species typically show less than 2% sequence divergence (corresponding with mid-Pleistocene differentiation) and have lower diversity values and little fine-scale phylogeographic structure. These differences can probably be generalized to include other glaciated regions as well, as species from glaciated regions typically have much lower levels of genetic diversity than species from nonglaciated areas (Billington and Hebert 1991).

Intraspecific phylogeographic patterns among freshwater fish species in the southeastern United States are strongly concordant, owing to similar effects of sea level changes and drainage patterns on each species (Bermingham and Avise 1986). Colonists emigrating from Gulf Coast and Atlantic refugia evidently had broadly similar dispersal patterns, with little to no secondary contact between refugial groups. These patterns are not only similar among marine, freshwater, and anadromous fish species, but also closely match phylogeographic data for bird, reptile, and marine invertebrate species (Avise 1992). In contrast, glacial disturbances and postglacial drainage connections were more extensive in the northern portion of the continent, resulting in qualitative differences in postglacial history and intraspecific phylogeographic patterns. Timing, extent, and conditions of proglacial lake connections were critical in determining modern distributions of freshwater species, resulting in more pronounced differences in phylogeographic structure among species owing to interspecific differences in thermal tolerance, habitat requirements, and dispersal abilities. Secondary contact among refugial groups was highly variable, with locations and extent of contact zones varying among species.

In conclusion, reconstructed dispersal events based on data from this and other studies indicate that both historical events and ecological characteristics of species play significant roles in determining the distribution and genetic structure of modern-day populations. The variation in postglacial origins and resultant genetic composition among species from eastern Canada and the northeastern United States and the lower regional concordance in comparison with other studies (e.g., Avise 1992) indicate that generalizations of phylogeographic history may be limited to ecologically equivalent groups or

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species. There is a clear need to examine more species with differing ecologies and distributions from key sites with known postglacial histories to better understand phylogeographic processes within this region.

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Conservation genetics of brook trout (*Salvelinus fontinalis*): population structuring in Fundy National Park, New Brunswick, and eastern Canada

Matthew W. Jones, Douglas Clay, and Roy G. Danzmann

Abstract: To determine the structure of brook trout (*Salvelinus fontinalis*) genetic variation in eastern Canada and to assess the potential impact of stocking in Fundy National Park, New Brunswick, 1096 and 445 brook trout were examined for allozyme and mitochondrial DNA variation, respectively. The trout were derived from 33 natural locations and one hatchery source. Low divergence among the mitochondrial DNA haplotypes suggests a single glacial refugium for the trout recolonizing the region. High levels of population differentiation were found with allozymes, with the majority of the differentiation occurring among branches within drainages of rivers, suggesting little present-day gene flow among most populations. Comparisons of both allozyme and mitochondrial DNA variation between descendants of the hatchery strain used to stock Fundy National Park and present-day populations within the park suggest that hatchery trout made minimal contributions to the gene pools of these populations. Lower levels of heterozygosity were found in brook trout from lakes than from streams, possibly because of intense fishing pressure.

Résumé : Pour déterminer la structure des variations génétiques de l'omble de fontaine dans l'est du Canada et évaluer l'impact potentiel de l'ensemencement dans le parc national de Fundy (Nouveau-Brunswick), on a examiné la variation des allozymes et de l'ADN mitochondrial chez 1096 et 445 ombles de fontaine (*Salvelinus fontinalis*), respectivement. Les poissons provenaient de 33 sites naturels et d'un élevage. La faible divergence parmi les haplotypes dans l'ADMmt semble indiquer que les ombles qui ont recolonisé la région provenaient d'un même refuge glaciaire. Des niveaux élevés de différenciation des populations ont été observés au chapitre des allozymes, la plus grande partie de cette différenciation se produisant entre les différentes branches des cours d'eau dans un même bassin-versant, ce qui permet de penser qu'il y a à l'heure actuelle peu de flux génétique entre la plupart des populations. Les comparaisons des variations des allozymes et de l'ADN mitochondrial entre les descendants de la souche d'élevage utilisée pour repeupler le parc national de Fundy et les populations actuellement présentes dans le parc permettent de penser que les poissons d'élevage n'ont contribué que de façon minime au patrimoine génétique de ces populations. Le niveau d'hétérozygotie observé était plus faible chez les ombles des lacs que chez ceux des cours d'eau, ce qui peut être dû à la plus forte pression de pêche.
[Traduit par la Rédaction]

Introduction

Conservation of a species should maintain the evolutionary potential of that species under natural environmental conditions. The evolutionary potential of a species may be defined in terms of its genetic variation (Frankel and Soulé 1981). Maintaining the genetic diversity of a species is recognized by managers of national parks throughout the world as being critical to conservation efforts (Anonymous 1984). Therefore, it is essential to quantify the amount and distribution of genetic variation within and among natural populations if they are to be properly managed and conserved (Ryman 1981).

For both economic and aesthetic reasons salmonid fishes represent one of the most important natural resources in North America. Brook trout (*Salvelinus fontinalis*), native to eastern North America, are now one of the most angled fish species in national parks throughout North America. As an example, brook trout were stocked in Fundy National Park, New Brunswick, intermittently in the 1930s and 1940s and extensively starting in the 1950s until 1981. Most of the stocked fish were placed in two lakes in the park. From 1956 to 1981, Bennett Lake received approximately 3200 trout per year (132 trout·ha^{-1}·year^{-1}), while Wolfe Lake received approximately 2000 trout per year (95 trout·ha^{-1}·year^{-1}) from 1958 to 1980. Accumulating evidence from several salmonid species suggests that when introgression of stocked fish with native fish occurs or when stocked fish affect native fish through competition, the performance of native stocks generally declines in comparison with that of unaffected populations (Hindar et al. 1991).

The major objective of our study was to characterize the genetic diversity of brook trout populations and their phylogenetic

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Fig. 1. Sampling sites of brook trout collected from the Canadian Maritimes. The outlined area indicates the location of Fundy National Park and is enlarged in Fig. 2. Site codes are defined in Table 1.

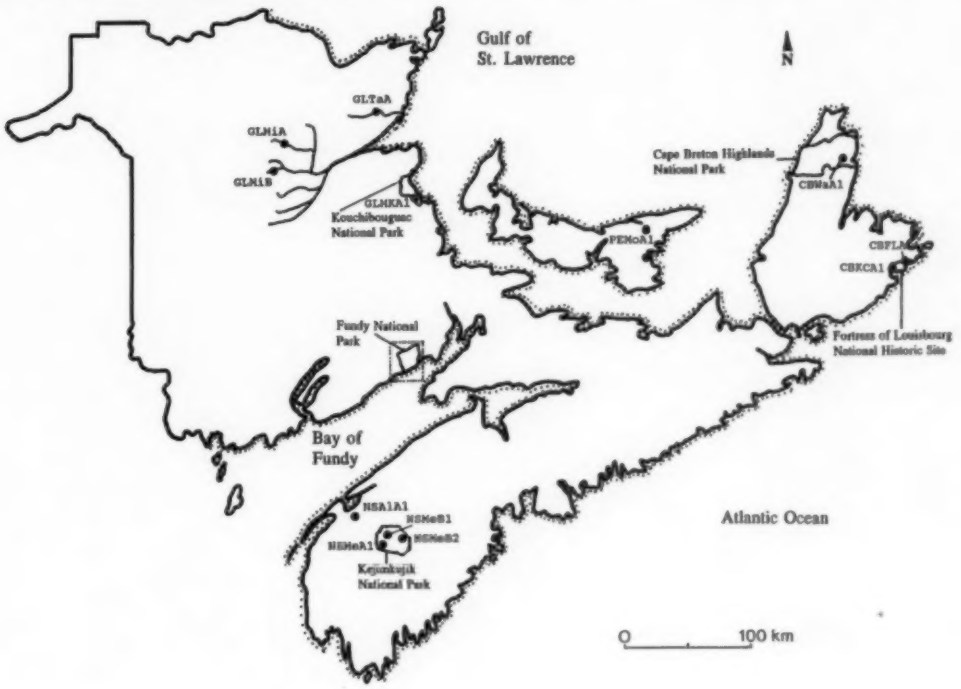
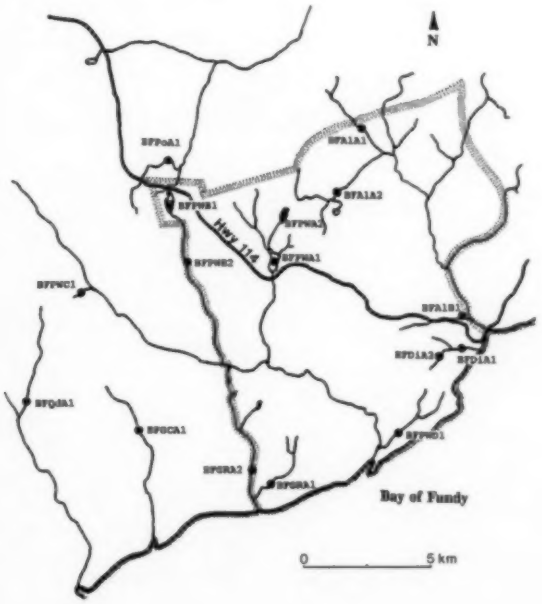


Fig. 2. Sampling sites of brook trout collected from Fundy National Park and adjacent drainages. Site codes are defined in Table 1.



relationships within five national parks in eastern Canada, with the most intense sampling effort concentrated in Fundy National Park, New Brunswick. Past stocking in Fundy National Park may already have had effects upon the genetic structure of locally adapted brook trout populations. Therefore, brook trout allozyme and mitochondrial DNA (mtDNA) variation from stocked and unstocked waters in and adjacent to Fundy National Park were compared to assess the impact of stocking on these populations. For these comparisons the last hatchery strain used for stocking in the park was genetically characterized.

Materials and methods

Collections

Brook trout were sampled over the summer and fall of 1992 and summer of 1993. Fish were obtained by electrofishing and angling or were collected in cooperation with personnel from the Department of Fisheries and Oceans (DFO) and the New Brunswick Department of Natural Resources and Energy (NBDNRE) at fish-counting fences. The sample of the Phillips hatchery strain, which originated in Maine, U.S.A., and was the last strain used for stocking in Fundy National Park, was obtained from the NBDNRE hatchery in Minto, N.B. Samples of brook trout were collected from one hatchery and 33 wild locations throughout the Maritimes (Figs. 1 and 2, Table 1). Stocking histories for the sites in the Fundy National Park region were obtained from the Scotia–Fundy regional office of the DFO in Halifax, N.S. Information was also obtained from Fundy National Park records (Hoar 1981) and NBDNRE (C. Ayer, NBDNRE, Fredericton, N.B., personal communication).

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Table 1. Region, drainage, assigned branch number, site code, year of sampling, and numbers of brook trout analysed.

Region	Drainage	Branch	Site	Site code	Year	N_D	N_a
W Nova Scotia	Mersey River	1	Lucifee Brook	NSMeA1	1992–1993	8	9
W Nova Scotia	Mersey River	2	West River	NSMeB1	1992	14	14
W Nova Scotia	Mersey River	2	Rogers Brook	NSMeB2	1992–1993	11	38
W Nova Scotia	Allain River	1	Allain River	NSAlA1	1993	8	20
Cape Breton	Warren Brook	1	Warren Brook	CBWaA1	1993	15	31
Cape Breton	Kennington Cove Brook	1	Kennington Cove Brook	CBKCA1	1993	13	14
Cape Breton	Fortress Estuary	1	Marconi Brook[a]	CBFLA1	1993	9	18
Cape Breton	Fortress Estuary	1	Fortress Brook[a]	CBFLA2	1993	7	15
Prince Edward Island	Morrell River	1	WBr. Morrell River	PEMoA1	1992	19	19
Gulf of St. Lawrence	Tabusintac River	1	Tabusintac River	GLTaA1	1992	22	12
Gulf of St. Lawrence	Tabusintac River	1	Powerline Brook[a]	GLTaA2	1993	33	33
Gulf of St. Lawrence	Major Kollock Brook	1	Major Kollock Brook	GLMKA1	1992–1993	15	31
Gulf of St. Lawrence	Miramichi River	1	NBr. NW Miramichi (LR)	GLMiA1	1992	13	13
Gulf of St. Lawrence	Miramichi River	1	NBr. NW Miramichi (UR)	GLMiA2	1993	15	45
Gulf of St. Lawrence	Miramichi River	2	Catamaran Brook (LR)	GLMiB1	1992–1993	17	17
Gulf of St. Lawrence	Miramichi River	2	Catamaran Brook (UR)	GLMiB2	1993	15	22
Gulf of St. Lawrence	Miramichi River	2	Otter Brook	GLMiB3	1992–1993	21	43
NE Bay of Fundy	Peticodiac River	1	Pollett River	BFPoA1	1992–1993	15	35
NE Bay of Fundy	Quiddy River	1	Quiddy River	BFQdA1	1992–1993	15	32
NE Bay of Fundy	Goose Creek	1	Goose Creek	BFGCA1	1992–1993	15	32
NE Bay of Fundy	Point Wolfe River	1	Bennett Lake	BFPWA1	1992–1993	8	73
NE Bay of Fundy	Point Wolfe River	1	Tracey Lake	BFPWA2	1992–1993	6	22
NE Bay of Fundy	Point Wolfe River	2	Wolfe Lake	BFPWB1	1992–1993	15	44
NE Bay of Fundy	Point Wolfe River	4	Hueston Brook	BFPWD1	1992–1993	20	50
NE Bay of Fundy	Point Wolfe River	2	East Branch	BFPWB2	1992	9	38
NE Bay of Fundy	Point Wolfe River	3	Sousbois Brook[a]	BFPWC1	1992–1993	10	30
NE Bay of Fundy	Upper Salmon – Alma River	2	Kinney Brook	BFAlB1	1992–1993	12	42
NE Bay of Fundy	Upper Salmon – Alma River	1	Broad River	BFAlA1	1992	7	54
NE Bay of Fundy	Upper Salmon – Alma River	1	Laverty Brook	BFAlA2	1992–1993	11	62
NE Bay of Fundy	Dickson Brook	1	Dickson Brook (LR)	BFDiA1	1992	12	12
NE Bay of Fundy	Dickson Brook	1	Dickson Brook (UR)	BFDiA2	1992	8	14
NE Bay of Fundy	Goose River	1	Rossiter Brook	BFGRA1	1993	11	64
NE Bay of Fundy	Goose River	1	Goose River	BFGRA2	1993	10	54
na	Hatchery (mixed)	na	Phillips strain	HatchP	1993	6	44
5 regions	16 drainages	22 branches	33 sites		Totals	445	1096

Note: The codes are as follows: the first two letters refer to region, the third and fourth letters to drainage, the fifth letter to branch designation, and the number to site. N_D, numbers of brook trout analysed for mtDNA; N_a, numbers of brook trout analysed for allozymes from each site; WBr., west branch; NBr., north branch; UR, upper reach; LR, lower reach.

[a]Names are assigned for convenience.

Electrophoretic analyses

Allozymes

Samples from the eye, liver, and white muscle were collected from freshly killed adult fish and stored in liquid nitrogen. Young of the year (YOY) were frozen whole and tissues sampled before analysis. Upon their arrival at the University of Guelph, tissues were transferred to a freezer (–80°C) until analysed for variation at 34 presumptive protein-coding loci. The following enzymes (abbreviation; EC number; tissues examined: E (eye), L (liver), and M (white muscle)) were screened for variation: aspartate aminotransferase (AAT; EC 2.6.1.1; E, L, M), aconitase (AH; EC 4.2.1.3; L), alcohol dehydrogenase (ADH; EC 1.1.1.1; L), adenylate kinase (AK; EC 2.7.4.3; M), creatine kinase (CK; EC 2.7.3.2; E, M), glycerol–3-phosphate isomerase (G3PDH; EC 1.1.1.8; M), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9; M), isocitrate dehydrogenase (IDHP; EC 1.1.1.42; L, M), L-lactate dehydrogenase (LDH; EC 1.1.1.27; E, L, M), malate dehydrogenase (MDH; EC 1.1.1.37; E, M), malic enzyme (MEP; EC 1.1.1.40; M), octanol dehydrogenase (ODH; EC 1.1.1.73; L), phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44; M), phosphoglucomutase (PGM; EC 5.4.2.2; M), and superoxide dismutase (SOD; EC 1.15.1.1; L). The protein products were resolved following the procedures described by Utter et al. (1974), Allendorf et al. (1977), and May et al. (1979) for 12% starch gels, and Hebert and Beaton (1989) for cellulose acetate gels. The genetic nomenclature for the protein-coding loci followed that recommended by Shaklee et al. (1990). Where possible, allele designations were standardized to those published (May et al. 1979, 1980; Stoneking et al. 1981; Johnson 1984; Perkins et al. 1993).

Mitochondrial DNA

Fresh heart and liver were placed in sterile TEKS buffer (50 mM Tris-HCl (pH 7.5), 10 mM ethylenediaminetetraacetic acid (EDTA), 0.20 M KCl, and 0.25 M sucrose). Buffer was not added to ovulated egg samples. Samples collected during the summer were shipped to the University of Guelph on wet ice for mtDNA extraction the following day. Samples collected during the fall of 1992 were kept on wet ice and processed upon return to Guelph. Mitochondrial DNA was extracted on the basis of the methodology described by Chapman and

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Powers (1984) and as modified by Danzmann et al. (1991*c*). This method yielded a partially purified mtDNA preparation.

The partially purified mtDNA was digested with restriction endonucleases. The 445 trout sampled for mtDNA analysis (see Table 1) were examined for the enzymes found to give variable restriction patterns in brook trout by Danzmann et al. (1991*b*, 1991*c*) and Danzmann and Ihssen (1995). Further polymorphisms were found by screening several fish from each population sampled in 1992; 54 individuals from these 25 populations were screened with the following 35 restriction enzymes: *Acc*I, *Alu*I, *Asn*I, *Asp*700I, *Ava*I, *Bam*HI, *Ban*I, *Ban*II, *Bgl*II, *Bst*XI, *Cfo*I, *Cla*I, *Csp*45I, *Dra*I, *Dra*II, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Hin*fI, *Hpa*II, *Ksp*I, *Mam*I, *Mbo*I, *Nco*I, *Nde*I, *Nhe*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Sna*BI, *Sph*I, *Xba*I, and *Xho*I. In addition, six individuals from the Maine Phillips hatchery strain were examined with 26 of these restriction enzymes. Total DNA extractions were used for these individuals, necessitating hybridization and probing to detect the mtDNA fragments; therefore, 10 of the less informative six-base restriction enzymes (*Bam*HI, *Bst*XI, *Csp*45I, *Dra*I, *Eco*RV, *Ksp*I, *Mam*I, *Pvu*II, *Sca*I, and *Xho*I) were omitted. The enzymes that were found to be variable (Table 4) were used to screen all of the trout in this study.

The mtDNA fragments were separated on 0.8–1.2% agarose (Boehringer-Mannheim) gels in TBE buffer and visualized by irradiation with ultraviolet light after ethidium bromide staining. DNA fragments of larger than 1 kilobase are generally observed using this technique. In those instances where ethidium bromide staining did not provide adequate resolution, DNA fragments were transferred from the gel onto a nylon membrane (Hybond N, Amersham Corp.) under vacuum (Pharmacia LKB VacuGene XL and Pharmacia LKB VacuGene Pump). The membranes were air dried, baked at 80°C for 1 h to overnight, and then probed with brook trout mtDNA labelled with digoxigen (Boehringer-Mannheim) using the random priming method of Feinberg and Vogelstein (1983). Probe was prepared from mtDNA extracted from fresh brook trout ova as described above. Detection was performed as described by Kessler et al. (1992) except lumigen PPD was used in place of the DPP solution and exposure time was increased to 16–48 h.

Statistical analyses

Allozymes

Intrapopulation analyses: Genotypic proportions were tested for agreement with Hardy–Weinberg expectations using χ^2 goodness of fit with Levene's (1949) correction for small sample size using BIOSYS-1 (Swofford and Selander 1981). *PGM-1** was excluded from this analysis owing to the presence of a null (ϕ) allele. It was not possible to distinguish between a non-ϕ allele homozygote (e.g., *100/100*) and a ϕ/non-ϕ allele heterozygote (e.g., ϕ/*100*). The *PGM-1** allele frequencies were determined assuming Hardy–Weinberg proportions. Linkage disequilibrium was estimated for each pair of loci in which the common allele had a frequency of less than 0.95 using G-Stat (Siegismund 1993). *PGM-1** and *sMDH-B1,2** were excluded. Diallelic loci were assumed for this analysis. In those few cases in which a locus had more than two alleles present, all combinations of alleles were made in which each allele or combination of alleles had a frequency greater than 0.05. Burrows' estimate of linkage disequilibrium, Δ (Weir and Cockerham 1979), was used to test the hypothesis that there was no linkage disequilibrium (i.e., $D = 0$) (see Siegismund 1993). Significance levels were adjusted using the Bonferroni procedure for populations with more than two polymorphic loci.

Interpopulation analyses: Mean observed and expected heterozygosities, based on Hardy–Weinberg expectations, were calculated for each population using BIOSYS-1 (Swofford and Selander 1981). The distribution of heterozygosities for individuals from the sampling locations was tested for normality using Lilliefors' test and their variances were tested for heterogeneity using Bartlett's test for homoscedasticity. Differences in observed mean heterozygosities were tested among the 33 locations using the Kruskal–Wallis test. Differences in observed mean heterozygosities between the lake and stream habitat type were tested for all locations and for the locations within the Point Wolfe River drainage (Fundy National Park) using the Mann–Whitney test.

A dendrogram was created using the neighbour-joining method (Saitou and Nei 1987) with Cavalli-Sforza and Edwards (1967) chord distance to examine the partitioning of genetic variation among sampling sites. This analysis was performed with PHYLIP version 3.57*c* (Felsenstein 1989) using all locations sampled and the hatchery strain. The neighbour-joining dendrogram was produced with MEGA (Kumar et al. 1993). Hierarchical *G* tests were used to determine if significant heterogeneity across loci existed between adjacent populations or groups of populations in the dendrogram.

To determine if there was an association between genetic and geographical distances, the matrix of Cavalli-Sforza and Edwards (1967) chord distances between all sampling locations was compared with the matrix of geographical distances between all sampling locations using the Mantel test (Mantel 1967).

All 33 wild sampling locations were hierarchically arranged into the five levels described in Table 1 to determine the level at which populations were differentiated. Explicitly, these levels were, from highest to lowest, (*i*) the total, (*ii*) regions within the total, (*iii*) river drainages within regions, (*iv*) river branches within river drainages, and (*v*) sites within river branches. Components of variance were estimated for each level on the basis of Wright's (1978) procedure for partitioning variance. Significance of the F_{XY} values was tested using a χ^2 statistic (Jordan et al. 1992). The hierarchical analysis was repeated excluding the eight populations from the Gulf of St. Lawrence region to determine if the genetic similarity among these populations affected the overall structuring of genetic variation. Although the populations from the other regions were sedentary, trout in several collections from this region (GLTaA1, GLMiA1, GLMiB1, and GLMiB3) had an anadromous or migratory life history.

Mitochondrial DNA

An analysis of molecular variance (AMOVA) using WINAMOVA (Excoffier et al. 1992) was conducted among the 33 wild sampling locations. Populations were arranged in three hierarchical levels: (*i*) the total, (*ii*) river drainages within the total, and (*iii*) sites within river drainages. Components of variance were estimated for each level as described by Excoffier et al. (1992).

Nucleotide site divergence (*d*) between all pairs of haplotypes was calculated using the method of Nei and Tajima (1981) and Nei and Miller (1990, eq. 4) using a presence–absence site matrix for the 23 polymorphic restriction sites. A total of 210 monomorphic sites (93 four-base and 117 six-base) were assumed present in the trout examined. This number was based upon the monomorphic fragments observed in the variable restriction enzymes as well as those that were monomorphic in the initial screen. Although not all fish were examined for all of these restriction sites, it was felt that their inclusion in the site matrix resulted in an accurate estimate of nucleotide divergence among these fish.

Certain multiple six-base recognition sequences were identical between some of the enzymes used (e.g., one site for *Hin*cII and *Acc*I). To compensate for these identical semi-isoschizomer sites, the total number of monomorphic sites detected for both enzymes was decreased by one eighth. Also, most of the four-base cutter sequences (i.e., from *Cfo*I, *Hpa*II, *Mbo*I, and *Alu*I) were recognized by internal sequences in one or more six-base cutting enzymes. Therefore, monomorphic sites detected by the four-base cutters were decreased to account for the overlaps with six-base sequences.

Genetic distances (*D*) among populations were calculated according to Danzmann et al. (1991*a*) using the MTDIS program. Standard errors were calculated after Nei and Tajima (1983) and Nei (1987,

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eqs. 5.41, 5.44, and 5.51). Nucleotide site divergence (d) between all pairs of haplotypes and their standard errors was calculated using the DSE program of REAP (McElroy et al. 1992). These calculated d values were used to estimate within-population nucleotide diversity (d_w; Nei 1987, eq. 10.19). Between-population nucleotide diversity (d_b) was calculated for all pairwise comparisons using eq. 10.20 of Nei (1987). Within- and between-population nucleotide diversities (d_w and d_b) were calculated using the DA program of REAP (McElroy et al. 1992).

A χ^2 Monte-Carlo bootstrapping program, CHI2MCS, using the pooled algorithm (Danzmann and Ihssen 1995) was used to assess significant differences in haplotype distributions in pairwise comparison between populations. Populations within drainages were first tested for differences. If they did not differ, they were combined for further analysis. The mtDNA D values among populations or groups of populations were clustered using the neighbour-joining method (Saitou and Nei 1987) with MEGA (Kumar et al. 1993).

Results

Allozymes

Variation was found at 11 of the 34 presumptive loci examined (*sAAT-4**, *ADH**, *G3PDH-1**, *sIDHP-1**, *LDH-A1**, *LDH-B1**, *LDH-B2**, *sMDH-B1,2**, *mMEP-1**, *mMEP-2**, *PGM-1**). The alternate allele for *mMEP-2** was never greater than 5%. The other 10 loci were polymorphic (common allele <95%) in at least one sampling location (certain individuals had three *120* alleles at the duplicated locus *sMDH-B1,2** in populations that contained individuals with three or four *100* alleles, suggesting that both loci may in fact be polymorphic. Although it is possible to test whether such variation occurs at two duplicated loci or a single tetrasomic locus (Waples 1988), we treated these loci as a single tetrasomic locus because high levels of variation were only observed in the Phillips strain of hatchery trout and the Quiddy River, New Brunswick, population). Variation was also observed at *sAAT-1,2** and *ODH**. However, they were not consistently resolved and were therefore not included. Allelic frequencies at the 11 variable loci for all locations sampled are given in Appendix 1. The observed phenotypes of the variable proteins and their genetic interpretations have been described elsewhere (Jones 1995).

Intrapopulation analysis

Tests for Hardy–Weinberg conformity

There were significant deviations from expected Hardy–Weinberg genotypic proportions in 11 of 165 tests in the 33 wild sites sampled. This was slightly greater than the 8.25 significant deviations that would be expected by chance at the 5% significance level. When significance levels for each test were adjusted to account for multiple testing (Bonferroni procedure (Weir 1990)), 7 of the 11 cases remained significant. However, in six of these seven cases, expected frequencies of some of the classes were low (i.e., <3). Only Marconi Brook had a class with a large (>5) expected distribution. Seven of the 18 trout sampled at Marconi Brook were YOY. A danger with sampling YOY is the possibility of individuals being from one or few matings. All four of the *sIDHP-1* 100/100* homozygotes were YOY, suggesting that this deviation from Hardy–Weinberg expectations was a result of a sampling bias.

Tests for allelic associations

There were 32 instances of linkage disequilibrium in 449 tests (22.5 expected by chance at the 5% level). When significance levels for each individual test were adjusted to account for multiple testing, only one significant association was found. In Fortress Brook (CBFLA2), *G3PDH-1** was in linkage disequilibrium with *sIDHP-1** ($\Delta = 0.057$, $p = 0.0031$ (critical $p = 0.0085$)). However, this result was likely an artifact of small sample size (15 individuals). One individual possessed an alternate allele for *sIDHP-1** (*100/100* homozygote). This individual was also one of three individuals from Fortress Brook found to be heterozygous at the *G3PDH-1** locus.

Interpopulation comparisons

Heterozygosity

The percentage of the 11 variable loci that were polymorphic for each population ranged from 27 to 73% (3–8 of 11 loci; Appendix 1). Mean levels of expected heterozygosity ranged from 0.091 to 0.279 (on the basis of the 11 variable loci; 0.029–0.090 over 34 loci). The observed and expected mean heterozygosity values for each population are given in Appendix 1. The lake populations (Bennett (BFPWA1), Tracey (BFPWA2), and Wolfe (BFPWB1) lakes, Fundy National Park) appeared to have lower levels of heterozygosity than all other populations. The heterozygosities of all populations were not normally distributed (Lilliefors' test) and their variances were heterogeneous (Bartlett's test for homoscedasticity, $\chi^2 = 115.054$, df = 31, $p < 0.001$). Therefore, population observed mean heterozygosities were tested for differences using the Kruskal–Wallis and Mann–Whitney tests.

There were highly significant differences among population mean heterozygosities. Also there was a significant effect of habitat type (lake versus stream). Stream fish were more heterozygous than lake fish. While there were still highly significant differences among population mean heterozygosities within the stream habitat type, there was no significant difference among the mean heterozygosities of the lake populations (Table 2). Repeating the analysis with only the populations within the Point Wolfe River drainage (the three lakes and Sousbois Brook, East Branch, and Hueston Brook) again revealed a significant effect of both population and habitat type (lake versus stream). There were no significant differences in mean heterozygosities among the three Point Wolfe stream populations (Table 2).

Cluster analysis

The neighbour-joining cluster analysis of Cavalli-Sforza and Edward's (1967) chord distance showed that populations within the same region generally cluster together (Fig. 3). For example, the populations within the Gulf of St. Lawrence were tightly clustered and the majority of the populations from the northeastern Bay of Fundy clustered together. The Quiddy River (BFQdA1) did not cluster with the other Bay of Fundy populations but rather with the Phillips hatchery. Populations from the two regions with fewer sampling locations, western Nova Scotia and Cape Breton Island, were less associated. For example, although the three populations from the Mersey River drainage (NSMeA1, NSMeB1, and NSMeB2) were clustered together, the fourth population from western Nova Scotia (Allain's Brook, NSAlA1) was not. Similarly, in the

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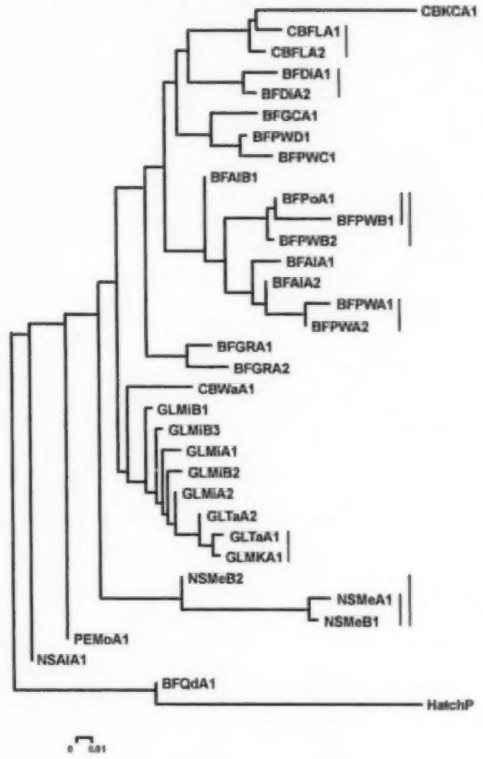
Table 2. Mean heterozygosity level differences among the 33 wild populations, between stream and lake populations, and among populations of streams and lakes.

Populations	n	Effect of:	*H*	df	*p*	*U*	χ^2	df	*p*
All	33	Population	332.4	31	<0.001	—	—	—	—
		Habitat	—	—	—	988 040	137.6	1	<0.001
Streams	30	Population	212.6	28	<0.001	—	—	—	—
Point Wolfe	6	Population	47.0	5	<0.001	—	—	—	—
		Habitat	—	—	—	11 781	42.1	1	<0.001
Lakes	3	Population	4.6	2	0.102	—	—	—	—
Streams	3	Population	1.2	2	0.536	—	—	—	—

Note: Tests were made using the variable loci with the exception of sMDH-B1,2* and PGM-1*. The Kruskal–Wallis test statistic (*H*), df, and *p* or the Mann–Whitney *U* statistic, with its chi-square approximation (χ^2), df, and *p* are given for each test. Mean observed heterozygosity values are given for each population in Appendix 1.

Fig. 3. Neighbour-joining dendrogram of the allozyme genetic distance (Cavalli-Sforza and Edwards 1967) calculated among brook trout samples from eastern Canada. Genetic distances are additive according to the scale shown and are based on the 11 variable loci. Site codes are defined in Table 1.



Cape Breton region, Warren Brook (CBWaA1) did not cluster with the other three populations from the region.

A Mantel test (Mantel 1967) of the correlation between matrices of genetic distance and geographical distance of all wild sampling locations was significant ($r = 0.325, p < 0.001$). This indicates that, overall, geographic proximity did correspond to genetic similarity, although this only accounts for 11% (r^2) of the association observed.

Hatchery differences

There were no loci with fixed differences in allele frequencies between the Phillips hatchery fish and the wild populations. The hatchery trout did possess alleles that were rarely found in the wild. The *sMDH-B1,2*120* allele (frequency of 0.24 in the hatchery trout) was at low (<0.03) frequency in six populations and higher in two (0.22 in BFQdA1 and 0.09 in NSAlA1). The *sIDHP-1*152* allele (frequency of 0.14 in the hatchery trout) was at low (<0.03) frequency in four populations and higher in BFQdA1 (0.09). The *LDH-B1*72* allele was in high frequency (0.90) in the hatchery trout. It was observed in low (<0.05) frequency in 10 populations and higher frequency in 7 (0.06 in NSMeA1, 0.10 in CBWaA1, 0.13 in GLTaA1, 0.14 in BFGRA2, 0.16 in BFQdA1, 0.24 in PEMoA1, and 0.33 in NSAlA1). The *PGM-1*60* allele was only observed in the hatchery trout (frequency of 0.27).

Hierarchical F *statistics*

The F_{XY} value for each hierarchical level was highly significant (Table 3), indicating significant structuring at each level. Of the total variance, 76.8% was found within sites. The largest proportion of the remaining variation (50.1%) was found between branches within drainages (Table 3). When the Gulf of St. Lawrence region, a region with a lower level of within-region population differentiation (Fig. 3; M.W. Jones, R.G. Danzmann, and D. Clay, unpublished results) was excluded from the analysis, higher F_{XY} values were obtained (e.g., $F_{ST} = 0.262$). However, the branch level remained the level with the highest proportion (56.4%) of the among-site variance.

Mitochondrial DNA analysis

Of the 35 restriction endonucleases examined, 14 produced variable fragment patterns (Table 4, Fig. 4). A total of 19 haplotypes were identified, with only 5 (haplotypes 1, 2, 6, 7, and 16) previously recorded (Danzmann et al. 1991*b*, 1991*c*; Bernatchez and Danzmann 1993; Danzmann and Ihssen 1995). Variant haplotypes were rare, with 87% (385 of 445) of the individuals having the common haplotype (i.e., haplotype 1; Appendix 2). Variant haplotypes were closely related to the common haplotype; 14 differed by only one restriction site (0.038% nucleotide site divergence from haplotype 1), 2 by two sites (0.076% nucleotide site divergence), 1 by three sites (0.115% nucleotide site divergence), and 1 by six sites (0.229% nucleotide site divergence) (Fig. 4). Among-population nucleotide diversity was low and ranged from 0 to

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Table 3. *F* statistics combined across loci for all populations and excluding the eight populations from the Gulf of Lawrence region.

Comparison		All populations			Excluding Gulf of St. Lawrence		
X	*Y*	Variance component	% of population variance	F_{XY}	Variance component	% of population variance	F_{XY}
Site	Total	0.600	100.0	0.232***	0.640	100.0	0.262***
Branch	Total	0.518	—	0.200***	0.521	—	0.213***
Drainage	Total	0.217	—	0.084***	0.160	—	0.065***
Region	Total	0.142	23.8	0.055***	0.064	9.9	0.026***
Site	Branch[a]	0.082	13.7	0.040***	0.119	18.7	0.062***
Branch	Drainage[b]	0.301	50.1	0.127***	0.361	56.4	0.158***
Drainage	Region[c]	0.075	12.4	0.030***	0.096	15.0	0.040***

Note: Hierarchical levels are described in Table 1. Significance levels calculated after Jordan et al. (1992): ***, $p < 0.005$.

[a] $F_{SB} = (F_{ST} - F_{BT})/(1 - F_{BT})$.

[b] $F_{BD} = (F_{BT} - F_{DT})/(1 - F_{DT})$

[c] $F_{DR} = (F_{DT} - F_{RT})/(1 - F_{RT})$.

1.084×10^{-3} (mean of 1.47×10^{-4}). Within-population nucleotide diversity was also low and ranged from 0 to 5.67×10^{-4} (mean of 8.80×10^{-5}). Complete details of mtDNA analyses can be found elsewhere (Jones 1995).

The mtDNA variants, though low in frequency, were generally of limited distribution. Of the haplotypes observed in the wild, five (2, 6, 16, 29, and 41) were found in only one individual. Six of the 12 alternate wild haplotypes occurring in more than one individual were unique to one sampling location (haplotypes 7, 35, 38, 43, 44, and 46), and 4 were unique to a drainage (haplotypes 37, 40, 45, and 49). The remaining two haplotypes (36 and 42) were each confined to small geographical areas (Appendix 2).

The AMOVA indicated little heterogeneity with respect to mtDNA genetic divergence (Table 5). Over 87% of the variance was found within populations, 10% among drainages, and the remaining 3% among populations within drainages. Nevertheless, significant differences were found among drainages in haplotypic composition (Fig. 5, Appendix 3). This is due to the fact that variant haplotypes were generally restricted to populations within drainages indicating their relatively recent origin and limited dispersal in these regions. Significant differences were not found among populations within drainages, and therefore populations were combined by drainage for the analysis.

The Phillips hatchery strain showed the greatest differentiation from all other populations; all six individuals sampled had a haplotype not observed in any other trout. The population from Kennington Cove (CBKCA1) in Cape Breton, the populations in the Tabusintac River drainage (GLTa) in the Gulf of St. Lawrence region, and populations from the Upper Salmon River drainage (BFAl) all differed significantly from all other populations. The populations from the southwestern Nova Scotia drainages (NSMe and NSAlA1) differed significantly from all other populations but did not differ from each other. Given the large number of pairwise comparisons performed, however, *p* values greater than 0.001 may be considered nonsignificant (Rice 1989).

Discussion

Genetic variation in streams and lakes

Lower levels of heterozygosity were found in three lake populations (Wolfe (BFPWB1), Bennett (BFPWA1), and Tracey (BFPWA2)) in the Point Wolfe drainage when compared with stream populations (Appendix 1, Table 2). This finding was unexpected. The lower levels of heterozygosity suggest lower effective population sizes (N_e) for these lakes than for the streams. Lower effective population sizes may result from a low number of founders and (or) population bottlenecks (Hartl and Clark 1989). However, at least for Bennett and Tracey lakes, immigration of brook trout from inlet streams may be expected to reduce the effect of such events. Approximately 60% of the preseason biomass is removed from these lakes annually by angling (Hoar 1981; Woodley 1990). There is little to no angling on the streams sampled. This fishing pressure could induce repeated population reductions and account for the lower heterozygosities observed for the lakes. Effective population size can also be affected by unequal numbers of males and females, overlapping generations, and a large variance in family size and dispersal ability (Hartl and Clark 1989). Differing demographics of populations in the two environments could play a role in affecting these factors. Similarly, greater environmental heterogeneity in the stream habitat could lead to selective advantages of increased heterozygosity in stream fish.

Another explanation for the low variation observed in lake populations may be the repeated stocking of hatchery trout. Bennett and Wolfe lakes always received trout from the same source hatchery and would therefore be expected to have similar alleles in high frequency at each locus, assuming that genetic drift was minimal. This was not the case for *ADH**. Wolfe Lake was nearly fixed for the common *-100* allele whereas Bennett and Tracey lakes were nearly fixed for the alternate *-205* allele. This suggests that genetic contributions by hatchery trout were minimal and do not account for the lower levels of variation observed in the lakes.

Structuring of genetic variation

High levels of population differentiation are common among nonmigratory salmonids (e.g., Allendorf and Leary 1988) and brook trout from the present study conform to this pattern. In the maritime locations surveyed, 23.2% of the allozyme variation was due to differences among populations, indicating a fragmented population structure. The general restriction of mtDNA haplotypes to single drainages also suggests a low level of gene flow among populations. A trend for species with a more fragmented distribution to have a greater degree of

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Table 4. Fragment sizes (kilobases) of the restriction endonucleases that generated variable restriction fragment lengths in Maritime brook trout mtDNA.

Restriction endonuclease	Fragment pattern	Size (kilobases)																					
*Acc*I	A	8.60	3.42	2.98	1.77	0.48	0.33																
	E	9.08	3.42	2.98	1.77	0.33																	
*Alu*I	A	1.26	1.14	0.84	0.60	0.58	0.55	0.46	0.41	0.40		0.38(2)	0.33	0.29	0.27	0.26	0.25	0.23	0.21	0.19	0.18	0.17	0.12
	B	1.26	1.14	0.84	0.78	0.60	0.58	0.55	0.46	0.41	0.40	0.38(2)	0.33	0.29	0.27	0.26	0.25	0.23	0.21	0.19	0.18	0.17	0.12
	C	1.36	1.26	0.84	0.60	0.58	0.55	0.46	0.41	0.40		0.3(2)	0.33	0.29	0.27	0.26	0.25	0.23	0.21	0.19	0.18	0.17	0.12
	D	1.26	1.14	1.02	0.84	0.60	0.58	0.55	0.46	0.41	0.40	0.38(2)	0.33	0.29	0.27	0.26	0.25	0.23	0.21	0.19	0.18	0.17	0.12
*Asn*I	A	3.43	2.02	1.87	1.75	1.65(2)	1.59	0.95	0.58	0.57													
	E	3.43	2.60	2.02	1.87	1.75	1.65	1.59	0.58	0.57													
	F	5.08	2.02	1.87	1.75	1.65	1.59	0.95	0.58	0.57													
*Asp*700I	A	7.53	5.11	3.37	0.95																		
	B	5.74	5.11	3.37	1.79	0.95																	
	C	7.53	5.11	2.73	0.95	0.64																	
*Ban*I	A	5.10	4.90	4.10	1.82	0.58	0.28																
	D	5.10	4.40	4.10	1.82	0.58	0.52	0.28															
*Ban*II	A	2.17	1.90	1.63	1.60	1.41(2)	1.24	1.09	0.87(2)	0.74													
	B	2.81	2.17	1.63	1.60	1.41(2)	1.24	1.09	0.87	0.74													
*Bgl*II	A	15.38	1.23	0.20																			
	B	9.62	5.76	1.23	0.20																		
*Cla*I	A	1 cut site																					
	B	No cut site																					
*Dra*II	A	6.28	2.30	2.03	1.88	1.10	0.69	0.61	0.385	0.30													
	B	5.73	2.30	2.03	1.88	1.10	0.69	0.61	0.55	0.385	0.30												
	C	4.47	2.30	2.03	1.88	1.81	1.10	0.69	0.61	0.385	0.30												
	D	6.28	4.33	1.88	1.10	0.69	0.61	0.385	0.30														
	E	3.87	2.41	2.30	2.03	1.88	1.10	0.69	0.61	0.385	0.30												
*Hinc*II	A	3.16(2)	2.95	2.34	1.72	0.96	0.79	0.63	0.22														
	B	3.16(2)	2.95	2.34	1.72	1.64	0.96	0.79	0.63	0.22													
	C	3.38	3.16	2.95	2.34	1.72	0.96	0.79	0.63														
*Hind*III	A	5.31	4.03	3.18	2.41	1.91																	
	B	7.76	4.03	3.18	1.91																		
*Nco*I	A	8.98	7.72	1.18																			
	B	8.98	4.40	3.32	1.18																		
*Nde*I	A	9.60	3.32	3.10	0.70	0.45																	
	B	9.60	3.32	3.10	1.15																		
*Nhe*I	A	7.70	6.40	3.30																			
	B	6.40(2)	3.30	1.30																			
*Pst*I	A	16.49	0.31																				
	B	12.40	4.09	0.31																			
*Sph*I	A	9.90	3.75	3.00																			
	B	9.90	6.75																				

Note: Fragment pattern letter designations are the same as the letters used in Fig. 4 indicating site gains and losses. (2) indicates a probable double fragment.

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Fig. 4. Parsimony network of site gains and losses among mtDNA haplotypes in eastern Canadian brook trout. Letter designations for each site gain and loss with the various restriction enzymes used are in reference to haplotype 1. The restriction fragment sizes for the various enzymes are given in Table 4.

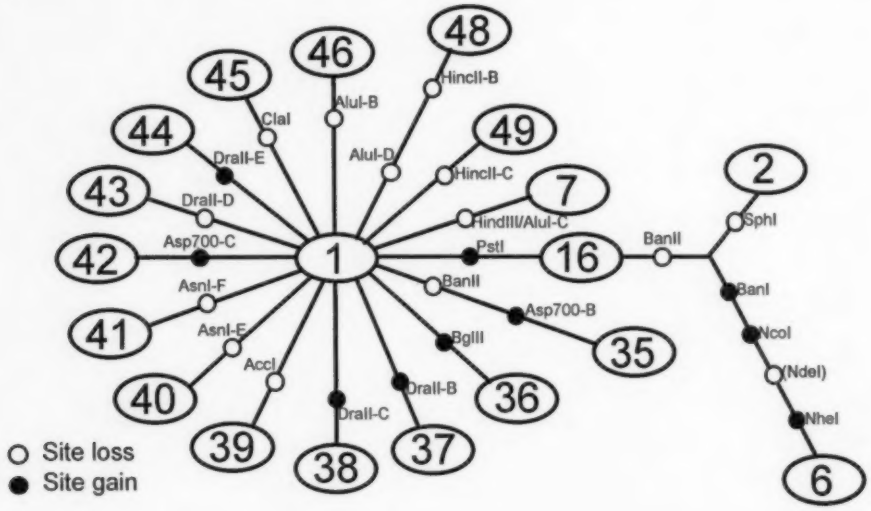
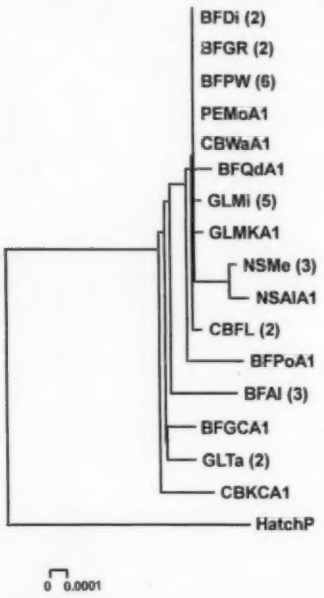


Table 5. Analysis of nucleotide diversity among and within brook trout populations in eastern Canada.

Variance component	Variance	% total	p^a	PHI statistics
Among drainages	0.015 024 9	10.16	0.009	PHI_{CT}=0.102
Among populations within drainages	0.003 834 1	2.59	0.033	PHI_{SC}=0.029
Within populations	0.129 046 8	87.25	<0.001	PHI_{ST}=0.128

[a]Probability of more extreme random values from 1000 permutation tests.

Fig. 5. Neighbour-joining dendrogram of the mtDNA genetic distances (*D*) among drainages within eastern Canada. Numbers in parentheses are the numbers of brook trout populations in the drainage. All distances are additive according to the percentage sequence divergence scale shown. Site codes are defined in Table 1.



population differentiation has been previously documented. Reviews of population structuring using allozyme data found that the average proportion of total intraspecific gene diversity allocated between locales generally increased from marine and anadromous to freshwater species, presumably owing to decreased gene flow among populations of freshwater versus anadromous and marine species (Gyllensten 1985; Ward et al. 1994). Avise (1992) made similar observations in reviewing mtDNA data for marine and freshwater species. The overall average proportion of total intraspecific allozyme gene diversity allocated between locales for the brook trout in this study (23.2%) is in the range for freshwater species. Similarly, the mtDNA localization index for this study is similar to those found for freshwater species. Interestingly, the average proportion of total intraspecific allozyme gene diversity allocated between locales for the brook trout from the Gulf of St. Lawrence region, where brook trout exhibit an anadromous life history, is 8.7%, within the range found for anadromous species (Gyllensten 1985; Ward et al. 1994; see Jones 1995; M.W. Jones, R.G. Danzmann, and D. Clay, unpublished results).

Hierarchical analysis of the allozyme variation suggests that much of the fragmentation of the Canadian maritime brook trout gene pool occurs at the level of branches within river drainages. Over 50% of the differentiation among sites is found at this level (Table 3). The lower contribution of the higher hierarchical levels (i.e., among regions and among drainages within regions) to the differentiation among sites is to be expected because the brook trout that recolonized the Canadian maritimes were likely of similar genetic composition.

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The low level of mtDNA variation within populations and the genetic similarity of the mtDNA haplotypes suggest that brook trout populations in eastern Canada originated from the same glacial refugium (Jones 1995). Therefore, regions and drainages contain brook trout of similar genetic composition for historical reasons. At a lower hierarchical level (branches within drainages), small numbers of founders, subsequent population bottlenecks, and random genetic drift in combination with restricted gene flow between branches likely all contributed to higher genetic differentiation. Some gene flow between sites within river branches could account for the lower level of differentiation at the lowest level. The similar findings when the Gulf of St. Lawrence region was excluded from the analysis indicate that the lower level of genetic differentiation within that region did not alter the overall genetic variation structure.

Failure of stocked brook trout in Fundy National Park

Our data suggest that the Phillips strain of brook trout stocked into Fundy National Park had little to no reproductive success. The Phillips strain differed in allele frequencies at several allozyme loci; alleles in high frequency in the Phillips strain were often rare (*sMDH-B1,2*120*, *sIDHP-1*152*, and *LDH-B1*72*) or absent (*PGM-1*60*) in the populations where stocking occurred (Appendix 1). Furthermore, the six Phillips strain individuals examined for mtDNA variation all possessed a unique haplotype not observed in any of the wild trout. The presence of these genetic markers in populations where hatchery fish had been stocked would be expected had the hatchery fish successfully reproduced.

Better survivorship of wild brook trout relative to planted hatchery strains has been previously reported (e.g., Danzmann and Ihssen (1995) and references therein). Artificial rearing of hatchery strains for generations results in their inability to adapt to natural conditions (Fraser 1989). This appears to be the case for the Phillips hatchery strain in Fundy National Park. The Phillips strain has been selected for fast growth and catchability for many generations, and it has low survivorship in the wild (F. Kircheis, Maine Department of Inland Fisheries and Wildlife, Bangor, Maine, personal communication). Observations of the ease with which Phillips strain brook trout were angled in Fundy National Park confirmed these reports (Woodley 1990). This suggests that most of the hatchery-released trout may have quite readily and rapidly been removed by angling.

Although most populations within the region show no evidence of genetic introgression with stocked trout, there is a population from one reportedly unstocked river that does show genetic similarities to the Phillips strain. The Quiddy River (BFQdA1) population and the Phillips hatchery strain (HatchP) (Fig. 3) have similar allele frequencies for several rare alleles that are in low frequency or are not present in other populations (i.e., *sMDH-B1,2*120*, *sIDHP-1*152*, and *LDH-B1*72*; see Appendix 1). This result is unexpected and is likely due to unrecorded stocking in this river and conditions existing that allowed for the introgression of the hatchery alleles.

Conservation and genetic variation

Stocking of brook trout in Fundy National Park could have led to the introgression of maladapted genes into native gene pools. However, the available evidence suggests that this did not occur. Therefore, brook trout in Fundy National Park appear to be representative of the trout historically adapted to the region. The failure of the Phillips strain to survive and reproduce also illustrates the importance of using locally adapted individuals in species reintroduction programs, which are unaltered by artificial rearing conditions.

The finding that the Fundy National Park lake populations have lower levels of genetic variation is of concern to the preservation of genetic variation within the park. Loss of genetic variation in these populations may lead to an inability to adapt to naturally changing environments. If this loss of variation can in part be attributed to high mortality from overfishing it would be appropriate to manage these lakes in a conservative manner, significantly reducing the numbers of trout removed.

The extent of population structuring in brook trout has implications, not only for the conservation and management of this species, but for the ability of national parks to conserve genetic variation of a species with limited ability to disperse. Although the structuring of the brook trout genetic variation is likely a reflection of their recolonization history, high levels of population differentiation exist among these trout, even in small geographical areas. The observed genetic differences are likely due to stochastic forces after recolonization. This does not mean that adaptive differences do not exist in each population (Leary et al. 1993). Indeed, the lack of gene flow among most populations would likely increase the rate of adaptation to their local environment. Therefore, while the brook trout of Fundy National Park have genetic variation representative of the region, they represent only a fraction of the genetic variation of the species. As Parks Canada policy recognizes (Anonymous 1994), national parks cannot conserve all biodiversity as though the parks are separate islands. This study reaffirms that conservation of biodiversity must occur in association with all adjacent land users.

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Appendix 1. Allelic frequencies, excluding the *100* (*-100* for *ADH**) allele, of the 11 variable loci in brook trout populations from eastern Canada.

Site	*sAAT-4**		*ADH**		*G3PDH-1**	*sIDHP-1**			*LDH-A2**	*LDH-B1**	*LDH-B2**
	*133	*170	*0	*–205	(*78)	*120	*140	*152	(*140)	(*72)	(*275)
NSMeA1	0.944	0.000	0.389	0.000	0.857	0.000	1.000	0.000	0.000	0.056	0.000
NSMeB1	0.769	0.000	0.536	0.000	0.714	0.000	1.000	0.000	0.000	0.000	0.000
NSMeB2	0.694	0.113	0.513	0.000	0.919	0.000	0.961	0.000	0.000	0.000	0.000
NSAlA1	0.500	0.167	0.725	0.000	0.850	0.050	0.925	0.000	0.000	0.325	0.000
CBWaA1	0.717	0.050	0.419	0.000	0.984	0.000	0.597	0.016	0.000	0.100	0.000
CBKCA1	1.000	0.000	0.964	0.000	0.964	0.000	0.393	0.000	0.000	0.000	0.000
CBFLA1	0.000	0.118	0.361	0.000	0.824	0.000	0.722	0.000	0.000	0.000	0.000
CBFLA2	0.000	0.036	0.300	0.000	0.900	0.000	0.933	0.000	0.000	0.000	0.000
PEMoA1	0.316	0.132	0.658	0.000	0.882	0.132	0.816	0.000	0.000	0.237	0.000
BFPoA1	0.400	0.571	0.357	0.000	0.986	0.000	1.000	0.000	0.000	0.000	0.000
BFQdA1	0.109	0.078	0.969	0.000	0.984	0.000	0.844	0.094	0.000	0.156	0.000
BFGCA1	0.016	0.375	0.156	0.000	1.000	0.188	0.813	0.000	0.000	0.000	0.000
GLTaA1	0.583	0.208	0.625	0.000	0.875	0.333	0.500	0.000	0.000	0.125	0.000
GLTaA2	0.652	0.106	0.682	0.000	0.848	0.242	0.591	0.000	0.000	0.048	0.000
GLMKA1	0.517	0.207	0.597	0.000	0.887	0.403	0.484	0.000	0.000	0.016	0.000
GLMiA1	0.769	0.154	0.692	0.000	1.000	0.038	0.885	0.000	0.000	0.000	0.000
GLMiA2	0.667	0.144	0.778	0.000	0.956	0.078	0.789	0.000	0.000	0.011	0.000
GLMiB1	0.618	0.206	0.559	0.000	1.000	0.059	0.706	0.029	0.000	0.000	0.000
GLMiB3	0.488	0.313	0.709	0.000	0.988	0.058	0.721	0.000	0.000	0.012	0.000
GLMiB2	0.600	0.300	0.682	0.000	0.977	0.091	0.636	0.023	0.000	0.023	0.000
HatchP	0.119	0.202	0.000	0.000	1.000	0.000	0.864	0.136	0.000	0.898	0.000
BFPWA1	0.036	0.833	0.918	0.000	1.000	0.007	0.993	0.000	0.000	0.000	0.021
BFPWA2	0.083	0.694	0.952	0.000	1.000	0.024	0.976	0.000	0.000	0.000	0.068
BFPWB1	0.304	0.679	0.085	0.000	0.951	0.000	1.000	0.000	0.000	0.012	0.000
BFPWD1	0.140	0.570	0.080	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000
BFPWB2	0.365	0.595	0.355	0.000	0.897	0.013	0.961	0.026	0.000	0.026	0.000
BFPWC1	0.433	0.367	0.017	0.000	1.000	0.000	1.000	0.000	0.000	0.017	0.000
BFAlB1	0.313	0.400	0.333	0.024	0.964	0.000	1.000	0.000	0.000	0.036	0.000
BFAlA1	0.042	0.417	0.509	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.028
BFAlA2	0.167	0.700	0.661	0.000	0.992	0.008	0.992	0.000	0.000	0.000	0.008
BFDiA1	0.000	0.375	0.500	0.000	1.000	0.000	1.000	0.000	0.000	0.042	0.000
BFDiA2	0.045	0.545	0.714	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000
BFGRA1	0.230	0.278	0.297	0.000	1.000	0.008	0.992	0.000	0.219	0.023	0.000
BFGRA2	0.567	0.144	0.434	0.000	1.000	0.038	0.962	0.000	0.274	0.135	0.000

Note: Sampling site codes are defined in Table 1. Observed and expected (Nei 1978) heterozygosity values (and standard errors) for these loci are given excluding *sMDH-B1,2** and *PGM-1**.

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*sMDH-B1,2**		*mMEP-1**	*mMEP-2**	*PGM-1**			Heterozygosity	
*120	*80	(*0)	(*50)	*ϕ	*140	*60	Obs (SE)	Exp (SE)
0.000	0.000	0.889	0.000	0.000	0.000	0.000	0.143 (0.062)	0.133 (0.057)
0.000	0.000	0.857	0.000	0.000	0.000	0.000	0.153 (0.074)	0.174 (0.072)
0.000	0.000	0.865	0.000	0.316	0.013	0.000	0.109 (0.046)	0.161 (0.068)
0.087	0.013	0.750	0.000	0.625	0.000	0.000	0.245 (0.074)	0.253 (0.077)
0.008	0.000	0.935	0.000	0.710	0.016	0.000	0.214 (0.082)	0.197 (0.073)
0.000	0.000	0.821	0.000	0.857	0.000	0.000	0.079 (0.042)	0.105 (0.059)
0.000	0.000	0.472	0.000	0.444	0.028	0.000	0.201 (0.083)	0.213 (0.073)
0.000	0.000	0.733	0.000	0.500	0.000	0.000	0.141 (0.071)	0.136 (0.058)
0.000	0.000	0.528	0.000	0.447	0.000	0.000	0.258 (0.074)	0.275 (0.078)
0.000	0.000	0.986	0.000	0.343	0.000	0.000	0.105 (0.065)	0.116 (0.072)
0.219	0.000	0.734	0.000	0.250	0.000	0.000	0.149 (0.054)	0.152 (0.054)
0.023	0.000	0.547	0.000	0.656	0.000	0.000	0.153 (0.064)	0.175 (0.074)
0.000	0.000	0.292	0.000	0.708	0.042	0.000	0.259 (0.079)	0.290 (0.086)
0.000	0.000	0.450	0.000	0.545	0.030	0.000	0.258 (0.087)	0.265 (0.082)
0.000	0.000	0.419	0.000	0.758	0.065	0.000	0.296 (0.105)	0.272 (0.092)
0.000	0.000	0.462	0.000	0.731	0.038	0.000	0.231 (0.103)	0.175 (0.074)
0.000	0.000	0.389	0.000	0.744	0.033	0.000	0.232 (0.087)	0.200 (0.073)
0.000	0.000	0.618	0.000	0.529	0.059	0.000	0.203 (0.084)	0.225 (0.089)
0.000	0.000	0.605	0.000	0.640	0.116	0.000	0.241 (0.095)	0.224 (0.087)
0.000	0.000	0.477	0.000	0.818	0.000	0.000	0.228 (0.085)	0.237 (0.087)
0.239	0.000	0.709	0.000	0.000	0.000	0.273	0.153 (0.068)	0.148 (0.065)
0.003	0.000	1.000	0.000	0.575	0.000	0.000	0.053 (0.032)	0.055 (0.034)
0.000	0.000	0.977	0.000	0.682	0.045	0.000	0.098 (0.059)	0.088 (0.051)
0.006	0.000	1.000	0.000	0.564	0.110	0.000	0.063 (0.037)	0.081 (0.050)
0.000	0.000	0.610	0.000	0.530	0.000	0.000	0.131 (0.077)	0.134 (0.077)
0.000	0.000	0.987	0.000	0.276	0.000	0.000	0.124 (0.056)	0.147 (0.068)
0.000	0.000	0.333	0.000	0.483	0.000	0.000	0.115 (0.074)	0.130 (0.081)
0.000	0.000	0.940	0.000	0.714	0.000	0.000	0.157 (0.081)	0.156 (0.082)
0.000	0.000	1.000	0.000	0.546	0.000	0.000	0.142 (0.089)	0.122 (0.076)
0.008	0.004	0.992	0.000	0.476	0.000	0.000	0.106 (0.064)	0.109 (0.066)
0.000	0.000	0.792	0.000	0.958	0.000	0.000	0.157 (0.073)	0.160 (0.075)
0.000	0.000	0.500	0.000	0.964	0.000	0.000	0.156 (0.079)	0.167 (0.084)
0.000	0.000	0.672	0.023	0.807	0.000	0.000	0.193 (0.074)	0.217 (0.081)
0.009	0.000	0.311	0.000	0.896	0.009	0.000	0.235 (0.077)	0.246 (0.079)

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Appendix 2. Distribution of mtDNA haplotypes in eastern Canada.

	Haplotypes																		
Site	1	2	6	7	16	35	36	37	38	39	40	41	42	43	44	45	46	48	49
NSMeA1	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NSMeB1	8	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—
NSMeB2	7	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—
NSAlA1	5	—	—	—	—	—	2	—	—	—	—	1	—	—	—	—	—	—	—
CBWaA1	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CBKCA1	8	—	—	—	—	—	—	—	—	—	—	—	2	—	3	—	—	—	—
CBFLA1	8	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
CBFLA2	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PEMoA1	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPoA1	13	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
BFQdA1	13	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFGCA1	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—
GLTaA1	16	—	—	—	—	—	—	—	—	—	1	—	—	—	—	5	—	—	—
GLTaA2	25	—	—	—	—	—	—	—	—	—	2	—	—	3	—	3	—	—	—
GLMKA1	14	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
GLMiA1	12	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
GLMiA2	13	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
GLMiB1	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GLMiB2	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GLMiB3	20	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
BFPWA1	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPWA2	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPWB1	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPWD1	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPWB2	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFPWC1	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFAlB1	8	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	1
BFAlA1	6	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFAlA2	7	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
BFDiA1	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFDiA2	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFGRA1	11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BFGRA2	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HatchP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—

Note: Site codes are defined in Table 1. Haplotype designations can be inferred from Fig. 4.

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Appendix 3. Monte-Carlo bootstrapping probabilities for pairwise comparisons of mtDNA haplotype counts among brook trout sampled from various drainages in eastern Canada.

	NSMe	NSAlA1	CBWaA1	CBKCA1	CBFL	PEMo	BFPo	BFQd	BFGC	GLTa	GLMK	GLMi	BFPW	BFA1	BFDi	BFGR
NSAlA	ns															
CBWaA1	*	*														
CBCKA1	***	*	**													
CBFL	*	*	ns	*												
PEMo	**	**	ns	***	ns											
BFPo	***	**	ns	**	ns	ns										
BFQd	**	*	ns	**	ns	ns	ns									
BFGC	***	**	ns	**	ns	ns	*	ns								
GLTa	***	***	*	***	*	*	**	**	***							
GLMK	*	*	ns	*	ns	ns	ns	ns	ns	*						
GLMi	***	***	ns	***	ns	***	**	**	***	ns						
BFPW	***	***	ns	***	ns	ns	***	*	***	***	ns	ns				
BFA1	***	***	*	***	**	**	***	**	***	***	*	***	***			
BFDi	**	**	ns	**	ns	ns	ns	ns	ns	*	ns	ns	ns	**		
BFGR	**	**	ns	***	ns	ns	ns	ns	ns	**	ns	ns	ns	**	ns	
HatchP	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

Note: Probabilities are based on 3000 replications. Site codes are defined in Table 1. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

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Long-term changes in zooplanktivorous fish community composition: implications for food webs

Timothy B. Johnson and James F. Kitchell

Abstract: The zooplanktivorous fish community of Lake Mendota has been dominated by cisco (*Coregonus artedi*), yellow perch (*Perca flavescens*), and white bass (*Morone chrysops*) for over 100 years. Shifts in their abundances have led to changes in zooplankton community composition. When total zooplanktivory was less than 0.5 g *Daphnia*·m^{-2}·day^{-1}, large-bodied *Daphnia pulicaria* dominated. At predation rates above 0.9 g *Daphnia*·m^{-2}·day^{-1}, *D. pulicaria* were rare. Large differences exist in the ability of each planktivore to regulate zooplankton community structure. In its lifetime, one cisco will consume as much *Daphnia* spp. as 14 yellow perch or 8 white bass. For equal biomass, cisco consume twice as much *Daphnia* spp. as do yellow perch or white bass. Almost 80% of the zooplanktivory by cisco is attributable to adults, while juvenile yellow perch and white bass account for over 50% of their species' predation. Cisco predation is highest during spring and fall and controls *D. pulicaria* population dynamics. Yellow perch and white bass predation is most intense during summer. When cisco biomass was less than 80 kg·ha^{-1}, *D. pulicaria* was the dominant zooplankter, regardless of yellow perch or white bass biomass. Continued improvements in water quality of Lake Mendota through biomanipulation require sustained low cisco biomass, enabling *D. pulicaria* populations to flourish.

Résumé : La communauté de poissons zooplanctonophages du lac Mendota est, depuis plus de 100 ans, dominée par le cisco de lac (*Coregonus artedi*), la perchaude (*Perca flavescens*) et le bar blanc (*Morone chrysops*). Les changements dans l'abondance de ces espèces ont occasionné des modifications de la composition de la communauté zooplanctonique. Quand la consommation totale de zooplancton était inférieure à 0,5 g *Daphnia*·m^{-2}·$jour^{-1}$, les *D. pulicaria* au corps massif dominaient. Quand le taux de prédation dépassait 0,9 g *Daphnia*·m^{-2}·$jour^{-1}$, les *D. pulicaria* étaient rares. Il existe de grandes différences dans la capacité de chaque planctonophage à réguler la structure de la communauté zooplanctonique. Au cours de sa vie, un cisco de lac va consommer autant de *Daphnia* que 14 perchaudes ou 8 bars blancs. À biomasse égale, le cisco consomme deux fois plus de *Daphnia* que la perchaude ou le bar blanc. Près de 80% de la consommation de zooplancton par le cisco est attribuable aux adultes, tandis que chez la perchaude et le bar blanc les juvéniles sont responsables de plus de 50% de la prédation. La prédation par le cisco est maximale au printemps et à l'automne, et régule la dynamique de la population de *D. pulicaria*. La prédation par la perchaude et le bar blanc est la plus intense pendant l'été. Quand la biomasse de cisco était inférieure à 80 kg·ha^{-1}, *D. pulicaria* était le zooplanctonte dominant, indépendamment de la biomasse de perchaude ou de bar blanc. La poursuite de l'amélioration de la qualité de l'eau du lac Mendota par biomanipulation nécessite le maintien d'une faible biomasse de cisco de lac, ce qui permettra aux populations de *D. pulicaria* de prospérer.
[Traduit par la Rédaction]

Introduction

Historically the zooplanktivorous fish community of Lake Mendota has been dominated by cisco (*Coregonus artedi*), yellow perch (*Perca flavescens*), and white bass (*Morone chrysops*) (Lathrop et al. 1992; Magnuson and Lathrop 1992). All three species have suffered massive and sometimes unexplained die-offs (Lathrop et al. 1992; Magnuson and Lathrop 1992), which when combined with variable recruitment have led to dramatic shifts in zooplanktivorous fish species composition during the last century. Similarly, the pelagic zooplankton community of Lake Mendota has undergone dramatic shifts in the relative proportions of its dominant species, *Daphnia pulicaria* and *Daphnia galeata mendotae* (Kitchell and Sanford 1992; Lathrop and Carpenter 1992). Large-bodied *D. pulicaria* are competitively superior to *D. galeata* (Lynch et al. 1986; Leibold 1989) and should dominate the zooplankton community in the absence of other controlling factors. However, larger zooplankton are selectively preyed upon by zooplanktivorous fish (Hrbacek et al. 1961; Brooks and Dodson 1965) such that smaller *D. galeata* should be favoured under conditions of high zooplanktivory (Rudstam et al. 1993).

Lake Mendota has been referred to as the most-studied lake in the world (Brock 1985). Extensive limnological and fish sampling has occurred at irregular intervals since the late 1800s (Kitchell and Sanford 1992; Lathrop and Carpenter 1992; Lathrop et al. 1992; Magnuson and Lathrop 1992). In this paper we combined these data sets with energetics models (Hewett and Johnson 1992) to explore interspecific, seasonal, ontogenetic, and interannual dynamics of zooplanktivory by cisco, yellow perch, and white bass. This analysis was then compared with estimates of *Daphnia* production to examine the effects of different zooplanktivorous fish on zooplankton

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population dynamics and species composition. Finally, we reconstructed the fish and zooplankton communities of Lake Mendota between 1880 and 1994 to compare interannual dynamics of fish predation with observed changes in the zooplankton community during the past century.

Methods

Historical trends in the fish community

Lathrop et al. (1992) and Magnuson and Lathrop (1992) provide comprehensive summaries of the long-term changes in the relative abundance of fishes in Lake Mendota. Using these published sources, unpublished reports, and dissertations, we quantified the zooplanktivore biomass (kilograms per hectare) of Lake Mendota between 1880 and 1994.

Much of the historical fishery data originated from gill nets; however, net design, location, and temporal (diel and seasonal) components of sampling have changed over time. We generated a standard catch per unit effort (CPUE) based on a multifilament vertical gill net set for 24 h in the late summer at the midlake station of Lake Mendota.

Cisco, yellow perch, and white bass spend the majority of their adult life in the open water of Lake Mendota (McNaught and Hasler 1961; McCarty 1990; Rudstam et al. 1993; Johnson 1995). As a result, only pelagic gill net sets (>10 m depth) were used to estimate CPUE, although alternative sampling techniques (creel surveys and angling records, inshore gill nets, fyke nets, and electrofishing) were used to characterize interannual variation in catch. We corrected for size selectivity associated with different mesh sizes on the basis of published retention probabilities (Berst 1961; Rudstam et al. 1984; Wilde 1991). Multifilament nylon nets (used after 1956) were considered to be twice as efficient as the cotton and linen nets they replaced (Berst 1961; Pycha 1962). The catch efficiency of monofilament nets was assumed to be 1.2 times that of multifilament nets (Larkins 1963). Because of reduced activity of all species during winter months (Becker 1983), gill-net CPUE was assumed to be one half of summer (June to September) values during the winter months (December through March) and linearly incremented during the spring and fall seasons. Yellow perch CPUE at night is approximately 10% of that during the daylight hours (Johnson 1995). The CPUE from gill-net sets of less than 24 h duration was adjusted in proportion to the number of hours set during dark and light periods. No diel correction was applied to white bass or cisco catches because these species do not exhibit the prominent diel activity pattern of yellow perch (Becker 1983; Johnson 1995). After standardization, gill-net CPUE was converted to fish biomass (kilograms per hectare) using established relationships (Rudstam and Johnson 1992; Johnson 1995; J.J. Magnuson, North Temperate Lakes Long-Term Ecological Research program, Center for Limnology, University of Wisconsin, Madison, WI 53706, unpublished data).

Historical trends in the zooplankton community

Long-term changes in the Lake Mendota zooplankton community were derived from direct measurement (summarized in Lathrop and Carpenter 1992; R.C. Lathrop, Wisconsin Department of Natural Resources, Bureau of Research, Madison, WI 53711, unpublished data). Total zooplankton density, as well as density of *D. pulicaria* and *D. galeata mendotae*, were standardized to numbers per square metre over a 20-m water column (Lathrop and Carpenter 1992). Biomass was computed using published length–mass regressions (Downing and Rigler 1984; Lynch et al. 1986). Paleolimnological analyses (Kitchell and Sanford 1992) were used to corroborate changes in the relative composition of the zooplankton community.

For the period between 1976 and 1994, birth and death rates of the dominant *Daphnia* spp. were calculated using the egg-ratio method (Paloheimo 1974). *Daphnia pulicaria* dominated the zooplankton community between 1976 and 1977, and between 1988 and 1994, while the smaller *D. galeata mendotae* was more abundant from 1978 to 1987 (Lathrop and Carpenter 1992). Weekly changes in density of the *Daphnia* spp. were used to estimate the instantaneous rate of increase (r) in the population. The average number of eggs per individual of each species (E) and the mean epilimnetic water temperature (T) were used to generate the per capita daily birth rate (b):

$$b = \frac{\ln(E+1)}{D}$$

and

$$D = \frac{1}{0.00041\,T^2 + 0.0108T - 0.0163}$$

where D is the development time (days) for natural populations of *Daphnia* spp. (Gabriel et al. 1987). Per capita daily mortality was derived by subtracting r from the estimated birth rate. Because all three zooplanktivorous fish species are size selective (Luecke et al. 1990; Johnson 1995), we corrected for size-selective mortality by using only large individuals (>1.6 mm for the years that *D. pulicaria* were dominant and >1.3 mm when *D. galeata* dominated) to generate the birth and death rates. Predation on *Daphnia* spp. by each of the zooplanktivorous fish was estimated using energetics models (described below) to determine the proportion of total *Daphnia* mortality explained by predation by each species of fish.

Energetics modelling

Energetics models (Hewett and Johnson 1992) were used to estimate predation by cisco (Rudstam et al. 1994), yellow perch (Kitchell et al. 1977; Post 1990), and white bass (Johnson 1995). The energetics models require species- and site-specific information on diet, energy density (calories per unit mass) of fish and prey, temperatures occupied by the fish, and growth rates. Prey consumption rates necessary to account for observed growth were calculated using an annual simulation period commencing January 1. Seasonal dynamics in consumption rates were calculated by accounting for seasonal variation in temperature, diet, and energy density (summarized below). In the absence of suitable energetics parameters for larval cisco, their predation was estimated using a gross conversion efficiency of 35% (Houde 1989; Arrhenius and Hansson 1993).

Temporal patterns in the diet of larval fish were estimated from weekly collections made using a fine-mesh purse seine (described in Post et al. 1992, 1995). The compositions of juvenile and adult cisco diets were determined for fish collected in vertical gill nets (described below) set between April and October of 1986–1993 at station depths of 6, 15, and 23 m. Extensive spatiotemporal analyses of juvenile and adult yellow perch and white bass diets were completed by Johnson (1995). Diets of all fish species are summarized in Table 1.

Seasonal energy densities of juvenile and adult fish were estimated from percent water content of tissues (Hartman and Brandt 1995; Johnson 1995), while larval trends were obtained from the literature (Table 2). Seasonal energy densities of most prey items were obtained from the work of Wissing and Hasler (1968, 1971) on Lake Mendota. The energy densities of copepods were estimated from Schindler et al. (1971).

We assumed that adult fish would be distributed in the water column close to their preferred temperature (15.8°C for cisco (Luecke et al. 1992*b*), 23°C for yellow perch (Kitchell et al. 1977), and 27.8°C for white bass (Reutter and Herdendorf 1976)). However, low hypolimnetic oxygen levels can force cisco into higher water temperatures during the summer and early fall (Rudstam and Magnuson 1985). We set the minimum oxygen threshold at 4 $mg \cdot L^{-1}$. These assumptions provide a good approximation for observed changes in the seasonal depth distribution of cisco and yellow perch in Lake Mendota (Luecke et al. 1990; Rudstam et al. 1993). The thermal histories of all larval fishes were described by temperatures recorded at 1 m. By midsummer the thermal distribution of young cisco is similar to that of the adults (MacKay 1963). Young of the year (YOY) white bass and yellow perch move from the pelagic zone as larvae into the

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Table 1. Seasonal changes in the percent composition by mass of prey in the diets of cisco (*Coregonus artedi*), yellow perch (*Perca flavescens*), and white bass (*Morone chrysops*) in Lake Mendota, Wisconsin.

		Daphnia		*Leptodora*		Copepoda		Nauplii		Amphipoda		Diptera		Other	
	Date	YOY	Older	YOY	Older	YOY	Older	YOY	Older	YOY	Older	YOY	Older	YOY	Older
Cisco	Jan. 1	na	100					na	0						
	Apr. 8	0	100			0	0	100	0						
	Apr. 22	10	100			90	0	0	0						
	June 22	90	100	0	0	10	0								
	Aug. 17	87	87	13	13	0	0								
	Oct. 12	65	65	35	35										
	Oct. 31	100	100	0	0										
	Dec. 31	100	100												
Yellow perch	Jan. 1	na	100			na	0	na	0						
	May 17	0	97			0	3	100	0	0	0	0	0	0	0
	June 22	12	93	0	0	88	1	0	0	0	2	0	3	0	1
	Aug. 17	55	65	5	5	0	0	0	0	20	20	15	5	5	5
	Oct. 12	70	84	5	16					15	0	10	0	0	0
	Oct. 31	90	100	0	0					5	0	5	0		
	Dec. 31	100	100							0	0	0	0		
White bass	Jan. 1	na	90			na	4	na	0	na	2	na	4	na	0
	June 22	0	54	0	0	0	0	100	0	0	5	0	38	0	3
	July 6	10	57	0	3	90	0	0	0	0	8	0	30	0	2
	Aug. 17	75	67	0	11	5	0			2	15	15	6	3	1
	Oct. 12	69	60	25	40	4	0			2	0	0	0	0	0
	Oct. 31	91	98	0	0	5	0			2	0	2	2		
	Dec. 31	90	90			4	4			2	2	4	4		

Note: The Diptera category includes both larvae and pupae, while the "other" category includes odonate nymphs, ephemeropteran nymphs, and fish. YOY, young-of-the-year; Older, all older age-classes. Blank cells indicate that prey type did not appear in the diet. na, not available as prey because the prey taxa were present prior to the appearance of first-feeding larvae.

littoral zone at a weight of approximately 0.1 g (Post et al. 1992) and remain there until the fall of their 1st and 2nd years, respectively (Luecke et al. 1990; McCarty 1990; Rudstam et al. 1993). We used surface water temperatures to describe the thermal history of yellow perch and white bass while distributed in the littoral zone.

Interannual variation in lake temperature

Interannual variation in lake thermal structure was incorporated into our energetics modelling using empirical and derived data. We summarized 48 years of temperature profiles collected on Lake Mendota between 1895 and 1994, selecting only years where no more than 14 days had elapsed between consecutive profiles during the ice-free period. We assumed that the lake was isothermal at a constant temperature of 3°C during the winter months. If vertical profiles of dissolved oxygen were not available, we used empirical relationships between the depth of the thermocline and the depth of low oxygen (Johnson 1995) to estimate the region below which <4 $mg \cdot L^{-1}$ oxygen was found. We used these observed thermal profiles to evaluate the importance of interannual variability in lake temperature on fish predation.

We also used empirical models developed for Lake Mendota to estimate lake thermal structure for years where no thermal profiles were available (Robertson 1989). Monthly air temperature (daily mean, minimum, and maximum), percent cloud cover, and wind velocity (average and maximum daily) data were corrected for sampling bias (Robertson 1989) and used to predict mean epilimnetic (surface to 7.5 m depth) and hypolimnetic (14.5–18.5 m depth) temperature. Biweekly water temperatures at the surface, at 1 m, at 2 m, and at the depth of low oxygen (<4 $mg \cdot L^{-1}$) were estimated from empirical relationships between date and epilimnetic and hypolimnetic water temperature (Johnson 1995).

Characteristics of the fish populations

Detailed growth rates of all zooplanktivorous fishes between 1981 and 1994 were obtained from scale analyses of fishes collected in vertical gill nets (4 m wide, 23 m deep, 19-, 25-, 32-, 38-, 51-,64-, 89-, and 127-mm stretched mesh) set as part of the Long-term Ecological Research North Temperate Lakes Project. Nets were suspended from the lake surface to 18 or 23 m depth at the midlake station (23 m) between late August and mid-September of each year. Additional samples were collected as part of the Lake Mendota Biomanipulation Project (T.B. Johnson, unpublished data).

When this growth rate information was combined with historical observations, we were able to identify five time intervals when adult growth rates showed different patterns. Perch growth rates were slow between 1880 and 1932 (Pearse and Achtenberg 1920; Bardach 1951). However, following the major die-offs of perch in 1929 and of cisco in 1932, growth rates increased (Bardach 1951). The 1940s were a period of rapidly increasing perch growth (Bardach 1951). Between 1951 and 1976, little interannual change was apparent in the growth rate of yellow perch (Bardach 1951; Rudstam et al. 1992); however, growth rates declined between 1977 and 1987 during the period of high cisco abundance (Johnson 1995). Subsequent to the cisco die-off in 1987 (Rudstam et al. 1993) perch growth rates returned to levels seen during the middle of this century. Therefore, yellow perch growth was characterized as slow (1880–1932), moderate (1933–1950 and 1977–1987), or rapid (1951–1976 and 1988–1994) (Table 3).

Much less information is available for growth rates of cisco and white bass. Comparison of cisco growth rates before and after the large cisco die-off in 1987 suggests that this species may experience density-dependent growth under high population conditions (Johnson 1995). Size at age for cisco in the late 1940s and early 1950s (John 1954) was comparable with the faster growing cisco collected in the late 1980s. We assumed that cisco growth was slow when they were abundant (1880–1932 and 1977–1987) and fast during other periods (1933–1976 and 1988–1994; Table 3). White bass size at age between

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Table 2. Seasonal changes in the energy density (cal·g wet mass^{-1}) of the principal zooplanktivorous fish in Lake Mendota, Wisconsin.

Age	Stage or date	Cisco	Yellow perch	White bass
0	First feeding	669[a]	1098[b]	743[c]
0	0.1 g	911[d]	550[e]	796[d]
0	1 g	1087[f]	943[d]	884[g]
0	Aug. 17	1301[d]	1158	1069
0	Sept. 12	1456	1186	1202
0	Oct. 12	1561	1225	1250
0	Dec. 31	1825	1150	1200
1	Jan. 01	1825	1150	1200
1	June 14	2197	1271	1289
1	Aug. 17	2125	1253	1226
1	Sept. 12	1812	1246	1323
1	Oct. 12	1815	1225	1323
1	Dec. 31	1825	1150	1250
2+	Jan. 01	1825	1150	1250
2+	Mar. 12	1900	1150	1242
2+	June 14	2197	1271	1289
2+	Aug. 17	2125	1253	1226
2+	Sept. 12	1812	1246	1323
2+	Oct. 12	1815	1225	1323
2+	Dec. 31	1825	1150	1250

Note: All values are from Johnson (1995) unless otherwise noted.
[a]Data are from Eldridge et al. (1977).
[b]Data are from Henderson and Ward (1978).
[c]Data are from Eldridge et al. (1982).
[d]Data are interpolated.
[e]Data are from Treasurer (1989).
[f]Data are from Gunkel (1981).
[g]Data from Wissing (1969).

1954 and 1964 (Horrall 1961; Voigtlander 1971) was nearly identical to that observed between 1981 and 1994. Because white bass were abundant during the earlier growth period and rare during much of the later period, we assumed their growth was constant for the entire record (Table 3).

Average growth rates of larval fish were estimated from weekly catches made with a fine- mesh purse seine between 1988 and 1993 (described in Post et al. 1992, 1995). Size of YOY fish was determined from mini-fyke net sampling conducted by the Wisconsin Department of Natural Resources between 1988 and 1993 (described in Johnson 1995). In the absence of additional data, we assumed that growth rates of larvae and of YOY fish were similar for each year.

All three species first spawn at age 3 with sex ratios of 1:1 (Becker 1983). Average loss of body mass (males and females combined) was 7.3% for cisco (Cahn 1927), 9.3% for perch (Craig 1977), and 6.5% for white bass (Ruelle 1977). Average date of spawning, hatch, and first feeding were related to lake temperature. Cisco spawn in the autumn when temperatures fall below 4°C (John 1954), hatch at ice-out, begin exogenous feeding 5 days later (John 1954; John and Hasler 1956), and attain a body mass of 0.1 and 1 g approximately 60 and 100 days after ice-out (1988–1993 average; T.B. Johnson, unpublished data). Yellow perch spawn in the spring after lake temperatures reach 9.1°C (Becker 1983). The eggs hatch 8–10 days later, and the larvae begin exogenous feeding in an additional 3–5 days (Becker 1983). Young yellow perch attain a body mass of 0.1 g 20 days after first feeding and 1 g 40 days later (1988–1993 average; T.B. Johnson, unpublished data). White bass spawn in the spring after lake temperatures reach 19.8°C (Horrall 1961). The eggs incubate for 2–4 days and the larvae begin exogenous feeding 4 days later (Ruelle 1971; Auer 1982). White bass larvae attain masses of 0.1 and 1 g, 15 and 40 days after initiation of exogenous feeding (1988–1993 average; T.B. Johnson, unpublished data).

Total adult mortality (fishing and natural mortality) was assumed to remain constant at 15% per year for cisco (Rudstam et al. 1993) and 50% per year for yellow perch (Johnson et al. 1992) and white bass (Horrall 1961; Johnson 1995). Annual mortality for age-0 and age-1 perch and white bass was 40%, while cisco mortality was 40% per year for age 0 and 25% per year for age 1. Fishing mortality was assumed to be negligible during the first 2 years (age 0 and age 1) for all fish. Mortality of all larval fishes was assumed to be 17.5% per day from hatch until the fish reached 0.1 g, 5% per day to 1 g, and 0.5% per day to September 15. These mortality schedules produce similar population dynamics to those described for larval yellow perch (Clady 1976; Treasurer 1989) and striped bass (*Morone saxatilis*, a close relative of white bass; Dey 1981) in other systems. No estimates of larval cisco mortality could be found in the literature.

Average life expectancy was 10 years for cisco and 5 years for yellow perch and white bass. We assumed a stable age distribution for all years, with the proportion by age determined by the mortality scenarios described above. The number of larvae and YOY fish were back-calculated from the number of age-1 fish for each year, using mortality rates described above.

Historical trends in zooplanktivory

Annual zooplanktivory rates for each species of fish were estimated using information on species biomass, growth rates, and age structure. Seasonal trends in fish diet and energy density of predator and prey were assumed constant throughout the simulations. Interannual variation in lake temperature was incorporated using the lake thermal profiles described above. For each year, a population of fish was generated using weight at age information specific to the growth rate scenario (low, moderate, or high; Table 3) and apportioning individuals amongst the ages to achieve the final target biomass.

Age-specific P values (the proportion of maximum consumption needed to achieve the observed growth) unique to each species and growth scenario were fit using the 48-year average temperature profile. For this P-fitting exercise, the annual individual mass increment was the difference between successive ages reported in Table 3. Annual predation was modelled by assigning P values and mass at age specific to the growth rate for that year. Individual predation for each age group was determined by allowing an individual of each age to grow from the initial mass assuming a constant P value. This assumption allowed annual growth rate to be sensitive to interannual variation in temperature. Total predation by each age group was calculated by multiplying daily individual consumption estimates by the population size (updated daily as per the mortality schedule). Total predation by the population was the sum of the predation rates determined for each age.

Annual predation rates were then compared with the zooplankton community structure (relative abundance of *D. pulicaria*) from the historical record. Estimates of fish predation and zooplankton community structure were both available from very few years (1917, 1959, 1969, and 1976 through 1994). However, these years represent a range of fish community compositions and abundances.

The assumptions made in developing these analyses are intended to minimize bias or error in developing a basis for long-term comparative studies. Because they are an acknowledgement of unknowns, we have attempted to make them conservative and to use simple interpolation in connecting observations. We have also adopted a conservative interpretation of results and confined our conclusions to those that derive from strong contrasts. As will become apparent, our results and their interpretation are robust and, therefore, not strongly influenced by the initial assumptions.

Results

Patterns in zooplanktivory

In its lifetime, one cisco will consume the same amount of

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Table 3. Average mass (g) at age and percentage of fish by age on January 1 under the different growth scenarios.

	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11
					Cisco						
Slow growth	59	225	321	397	448	484	517	548	577	606	632
Rapid growth	64	288	413	476	552	634	714	776	837	873	931
% at age	21	15	13	11	10	8	7	6	5	4	
					Yellow perch						
Slow growth	8	32	61	87	110	125					
Moderate	17	67	103	137	163	185					
Rapid growth	22	95	148	181	209	237					
% at age	47	28	14	7	4						
					White bass						
Growth	30	128	209	277	338	389					
% at age	47	28	14	7	4						

Note: Slow, moderate, and rapid refer to growth periods identified in the Methods.

Fig. 1. (A) Individual lifetime consumption (kg) of *Daphnia* spp. by cisco, yellow perch, and white bass. (B) Estimated predation on *Daphnia* spp. by standardized populations (50 kg·ha^{-1} for each species) of cisco, yellow perch, and white bass under long-term average conditions in Lake Mendota.

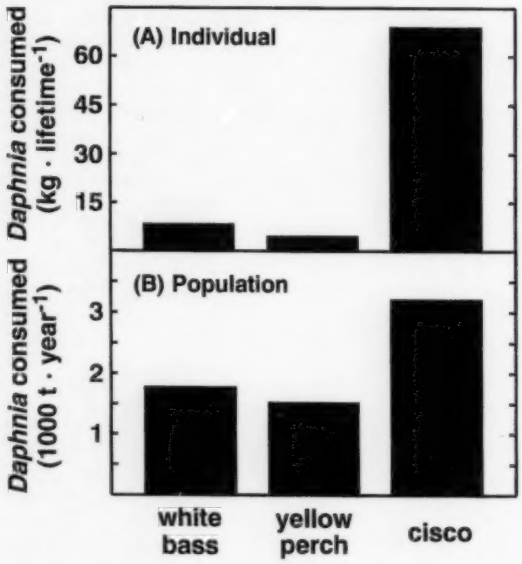
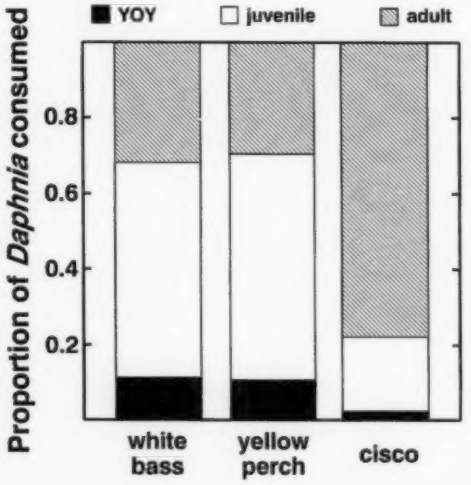


Fig. 2. Consumption of *Daphnia* spp. by selected age groups of cisco, yellow perch, and white bass in Lake Mendota. Standardized populations (50 kg·ha^{-1} for each species) composed of expected numbers of YOY, juvenile, and adult fish were simulated using long-term average growth and temperature conditions.



Daphnia spp. as 14 yellow perch or 8 white bass (Fig. 1A). To remove any effects of interspecific differences in diet, we also modelled predation assuming each species exclusively ate *Daphnia* spp. for its entire life. The result was essentially unchanged; one cisco will consume almost 12 times the amount of *Daphnia* spp. as one yellow perch, and 5.5 times that of a white bass. To remove any effects of interspecific differences in body size and longevity we used a standardized age-structured population of each zooplanktivore at equivalent biomass (50 kg·ha^{-1}). Even with this standardized population, cisco consumed twice as much *Daphnia* spp. in a year as did either of the other zooplanktivores under average temperature conditions (Fig. 1B). Within the standardized population, juvenile yellow perch and white bass accounted for over 50% of their species' total annual zooplanktivory, while adult cisco accounted for almost 80% of their species' total (Fig. 2). The YOY of each species accounted for 10% or less of the annual consumption of *Daphnia* spp.

Seasonally, predation on *Daphnia* spp. peaked in the late summer, being lowest during the winter months (Fig. 3). However, intraspecific and ontogenetic differences in the intensity of fish predation did occur. Cisco predation increased rapidly following ice-out, reaching an annual maximum in late June. By early to middle July, cisco predation reached a summer minimum as warm temperatures become unfavourable to this cold-water species. Predation by the warmwater species, white bass, increased more slowly in the spring, and reached a maximum in late August in correspondence with warm summer temperatures. Predation effects associated with recruitment of YOY perch and white bass cohorts were most notable in September and October, the same period when juvenile and adult

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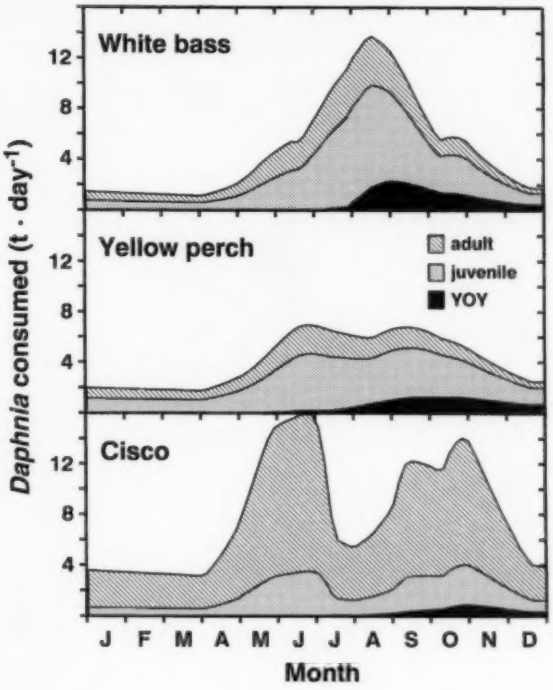
Fig. 3. Seasonal dynamics in the predation on *Daphnia* spp. by standardized populations (50 kg·ha^{-1} for each species) of cisco, yellow perch, and white bass in Lake Mendota. Long-term average growth and temperature conditions were assumed.

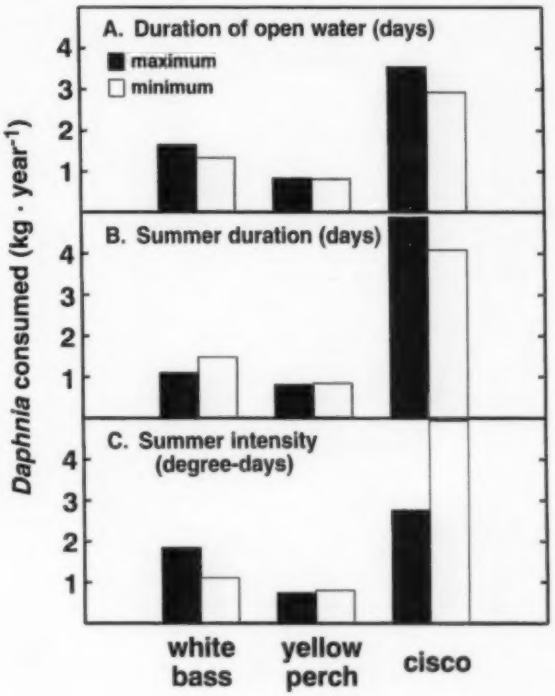


predation was declining. Cisco predation increased in September and remained high until middle to late November. Predation by all life stages of all species was lowest during the winter months.

Population-level predation by cisco was only modestly affected by variation in individual growth rates; however, yellow perch predation was sensitive to different growth rate scenarios. When a population of yellow perch experiencing slow growth was compared with one having the same total biomass, but growing under the fast growth scenario, consumption of *Daphnia* spp. was increased by 16%. This same, slow-growing population consumed 11% more *Daphnia* spp. than perch experiencing moderate growth. Increased consumption for slow-growing populations resulted from the 180 and 250% increases in numbers of moderate-and fast-growing individuals required to achieve the same population biomass of slow-growing individuals.

Interannual differences in lake temperature can have large effects on annual zooplanktivory by the species. The longest open-water period (1987, 296 days) was over 2 months longer than the shortest (1972, 232 days). Cisco and white bass predation both decreased by over 20% for the long versus short growing seasons, while yellow perch predation decreased by only 4% (Fig. 4A). A long, cool spring (1915, 187 days) can increase cisco predation by 22% relative to a short, warm spring (1962, 140 days). Yellow perch and white bass predation would be expected to decline by 4 and 25%, respectively, under similar spring conditions (Fig. 4B).

Fig. 4. The effect of interannual differences in thermal conditions on *Daphnia* predation by individual juvenile cisco, yellow perch, and white bass in Lake Mendota. The duration of open water is the number of days from ice-out in the spring to freeze up in the fall. Spring duration is the number of days from ice-out until the lake surface temperature exceeded 20°C for 3 consecutive days. Summer intensity is the cumulative number of degree-days greater than 17°C. For simplicity, we contrast the extreme years for each index.



Interannual differences in summer conditions produced the largest differences in predation by cisco and white bass. A long, hot summer (1921, 1017 degree-days) relative to a short, cool one (1915, 344 degree-days) cut cisco predation in half, reduced perch predation by 8%, and increased white bass predation by nearly 70% (Fig. 4C).

Fish regulation of zooplankton production

Between 1978 and 1987, almost all mortality estimated for the population of the dominant *Daphnia* spp. could be explained by fish predation. Cisco predation alone explained the majority of the observed *Daphnia* mortality. In the years preceding and following the domination by the 1977 cisco year-class, cisco predation was much less important in regulating *Daphnia* population dynamics. Yellow perch predation effects were more variable, although they too could account for much of the estimated mortality during the winter season of many years. Predation by yellow perch was also a major source of mortality during the fall of 1988 and the winter of 1993. White bass predation rarely accounted for more than 20% of the estimated *Daphnia* mortality in any year or season.

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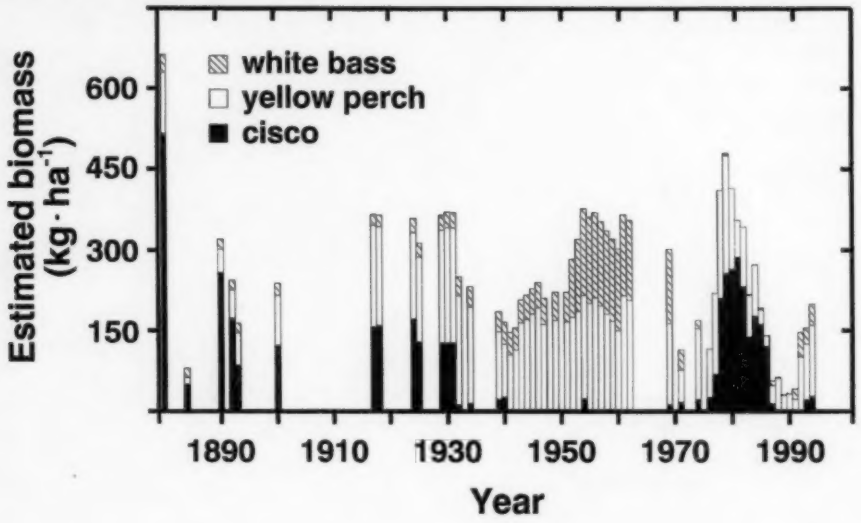
Fig. 5. Estimated biomass (kg·ha^{-1}) of the principal zooplanktivorous fishes in Lake Mendota between 1880 and 1994.

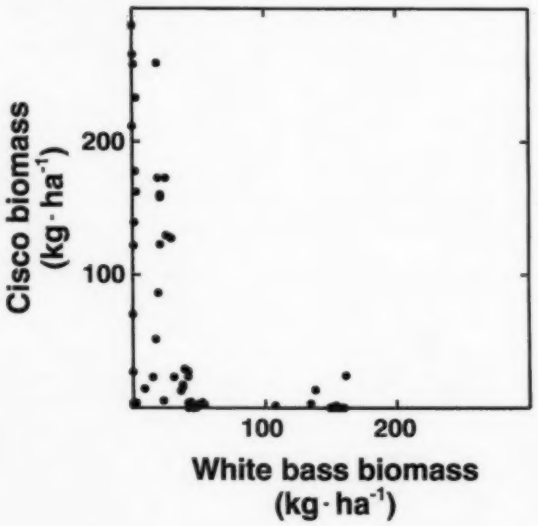


Changes in fish abundance

Total biomass of cisco, yellow perch, and white bass in Lake Mendota has varied between 200 and 400 kg·ha^{-1} for much of the century (Fig. 5). Massive cisco die-offs in 1884, 1932, 1940, and 1987 reduced cisco biomass by approximately 90% (Vanni et al. 1990; Lathrop et al. 1992; Luecke et al. 1992*b*). After the 1940 die-off, the abundance of cisco was so low that they were thought to have been extirpated from the lake (Lathrop et al. 1992). The fish epidemic of 1884 also killed large numbers of yellow perch and white bass (Forbes 1888). Except for periods of die-offs, total biomass of yellow perch has been near 180 kg·ha^{-1} during much of the record (Fig. 5). Small size at the turn of the century (Pearse and Achtenberg 1920; Bardach 1951) and poor recruitment in recent years (Rudstam et al. 1993) produced lower biomass estimates during these times. White bass biomass remained between 20 and 50 kg·ha^{-1} until the early 1950s when consecutive strong year-classes led to a rapid increase in numbers. Biomass remained near 150 kg·ha^{-1} through much of the 1950s and 1960s and then decreased gradually until the large die-off of 1976 (Lathrop et al. 1992). White bass biomass remained low through the 1980s; however, a large year-class recruited in 1991 and white bass biomass has increased to approximately 40 kg·ha^{-1} in recent years.

In general, the total biomasses of cisco and white bass are inversely related (Fig. 6). Cisco were abundant until the early 1930s and again between 1977 and 1986 whereas white bass were abundant between 1950 and 1970 and since 1991. Yellow perch are intermediate to cisco and white bass in terms of thermal preference and distribution, and their population trends showed no correlation with either species. In the absence of detailed growth and diet data, evidence of compensation between cisco and white bass is difficult to assess. However, extensive lags between cisco and white bass abundance shifts, combined with the lack of a yellow perch response, suggest that any underlying mechanism influencing the association between cisco and white bass abundance may have been obscured.

Fig. 6. Cisco and white bass biomass (kg·ha^{-1}) in Lake Mendota between 1880 and 1994.



Changes in zooplankton abundance

Prior to 1900, *D. galeata* constituted more than 95% of the pelagic daphnid fossils found in a core of Lake Mendota sediments (Kitchell and Sanford 1992). While the paleolimnological record indicated an increase in the proportion of *D. pulicaria* early in the 20th century, plankton samples collected by Birge and Juday between 1906 and 1917 showed a continued dominance by *D. galeata* (Lathrop and Carpenter 1992). Between 1940 and 1960, both the paleolimnological

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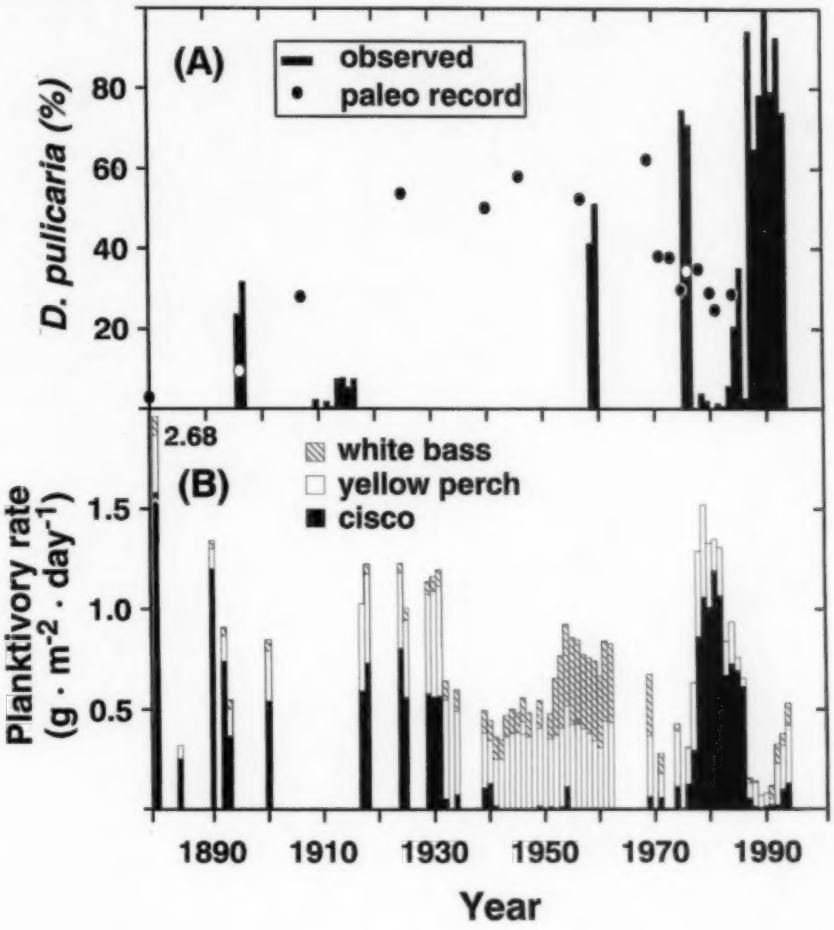
Fig. 7. (A) The percentage by number of *Daphnia pulicaria* relative to other herbivorous cladoceran species occurring in the pelagic region of Lake Mendota between 1880 and 1994. (B) Estimated zooplanktivory rate by cisco, yellow perch, and white bass in Lake Mendota between 1880 and 1994 as determined by energetics models.



record (Kitchell and Sanford 1992) and plankton samples (McNaught and Hasler 1964) indicated nearly equal proportions of *D. pulicaria* and *D. galeata*. Intensive zooplankton collections initiated in 1976 revealed that *D. pulicaria* constituted 75% of the annual daphnid abundance. However, between 1978 and 1987 *D. pulicaria* never constituted more than 5% of the daphnid abundance, except for brief periods in the springs of 1985 and 1986 (Lathrop and Carpenter 1992). Since 1988, *D. pulicaria* has constituted between 65 and 99% of the annual abundance of *Daphnia* spp. in Lake Mendota (Fig. 7A). The lack of correspondence between the observed and paleolimnological record in recent years is largely a consequence of sediment mixing owing to physical and biological processes, which have integrated several years of information in each core slice (Kitchell and Sanford 1992).

Interrelationships between zooplanktivory and *Daphnia* species composition

High fish biomass yielded high zooplanktivory rates (Fig. 7B). Zooplanktivory was highest prior to 1930 and between 1977 and 1987 when cisco were most abundant. Maximum cisco predation was over 2 g *Daphnia*·m^{-2}·day^{-1} preceding the 1884 die-off and had remained above 0.6 g·m^{-2}·day^{-1} through the 1930s and again in the early 1980s. Even during their most abundant period in the late 1950s, white bass consumed less than 0.5 g *Daphnia*·m^{-2}·day^{-1}. Yellow perch predation has ranged between 0.2 and 0.5 g *Daphnia*·m^{-2}·day^{-1} for most of the century.

Change in the composition of *Daphnia* spp. is not linearly related to zooplanktivory rates. In fact, a logistic regression best described the strong negative relationship between total zooplanktivory and the percentage of *D. pulicaria* in the zooplankton community (Fig. 8). When total zooplanktivory was less than 0.5 g·m^{-2}·day^{-1}, *D. pulicaria* dominated the zooplankton community. When total zooplanktivory exceeded 0.9 g·m^{-2}·day^{-1} *D. pulicaria* were extremely rare. Between these two levels of zooplanktivory lies a region of rapidly changing zooplankton community structure. As a result, few

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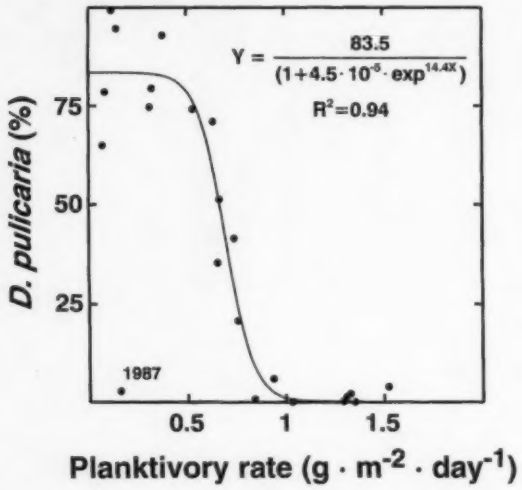
Fig. 8. The relationship between total zooplanktivory (g *Daphnia*·m^{-2}·day^{-1}) and the percentage of *D. pulicaria* relative to total cladoceran abundance in Lake Mendota between 1880 and 1994. The 1987 data point is an expected outlier because fish biomass (and thus planktivory rate) was estimated after the cisco die-off in August of 1987, while much of the zooplankton data were obtained prior to the die-off. This point was omitted when fitting the relationship.



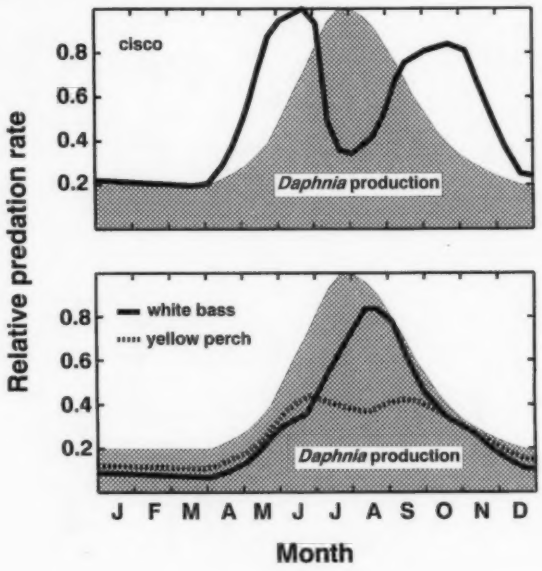
detailed inferences could be made associating fish and zooplankton community composition. At the turn of the century, *D. pulicaria* constituted less than 10% of the zooplankton community (Lathrop and Carpenter 1992), suggesting that fish predation exceeded 0.9 g·m^{-2}·day^{-1} throughout this period. This value was similar to estimated zooplanktivory in the early 1890s and again in the 1920s, suggesting that no major shifts in the zooplanktivorous fish community occurred during that 30-year period. Similarly, zooplanktivory estimates between the late 1920s and early 1960s suggest that *D. pulicaria* constituted between 50 and 70% of the zooplankton community after the cisco die-off in 1932. Evidence from the paleolimnological record supported these conclusions.

Discussion

In Lake Mendota, the zooplankton community is almost exclusively *D. pulicaria* when fish predation is less than 0.5 g *Daphnia*·m^{-2}·day^{-1}, whereas *D. galeata* dominates and *D. pulicaria* is virtually absent at predation rates greater than 0.9 g·m^{-2}·day^{-1}. Further, interspecific differences in predation by fishes can produce large interannual differences in total zooplanktivory. While yellow perch and white bass biomass often equaled or exceeded cisco biomass during much of the century, neither of these species seem to be able to regulate *D. pulicaria* populations as cisco have. Similar conclusions have been drawn using paleolimnological (Kitchell and Sanford 1992), historical (Magnuson and Lathrop 1992), and modelling efforts (Luecke et al. 1990, 1992*a*, 1992*b*); the cisco population is the primary regulator of daphnid species composition in Lake Mendota.

While high cisco abundance may explain the scarcity of

Fig. 9. Annual trends in the relative production of *Daphnia* spp. and the predation rates of cisco, yellow perch, and white bass in Lake Mendota in an average year.



D. pulicaria at the turn of the century and again in the 1980s, distinct physiological differences in zooplankton and fish may also contribute to the cisco's ability to regulate zooplankton community composition on an annual basis. At low zooplanktivory rates, *D. pulicaria* populations increase earlier in spring and outcompete *D. galeata* owing to physiological differences in population growth rates and filtering abilities (Threlkeld 1980; Leibold 1989). However, high cisco predation shortly after ice-out enables them to constrain *D. pulicaria* population growth rates at a time when warmer water species such as yellow perch and white bass cannot (Fig. 9). Perch and white bass predation increases later in the spring, after the *D. pulicaria* population has undergone its rapid growth. Once the zooplankton population reaches high abundance, regulation by fish predation is unlikely (Luecke et al. 1990, 1992*b*). Higher temperatures later in the year favour the life history of *D. galeata*, which may dominate in the late summer and early autumn. Lower susceptibility to size-selective predation and later development favour *D. galeata* under conditions of high zooplanktivory, while *D. pulicaria* will dominate in years of low cisco predation. When perch and white bass predation is high, *D. pulicaria* should dominate the spring, while *D. galeata* will dominate later in the year, and the two species are more equally represented in the total annual record. Zooplankton population dynamics recorded during the past century on Lake Mendota directly correspond with these mechanistic explanations.

Interspecific differences in temperature tolerance of the fishes may also explain the variable population dynamics of cisco and white bass (Lathrop et al. 1992; Magnuson and Lathrop 1992). Lake Mendota lies near the southern limit of the geographic range of cisco and near the northern limit of the white bass range (Scott and Crossman 1973; Lee et al.

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1980; Becker 1983). Conditions that are good for white bass growth and survival (i.e., warm summer conditions) are unfavourable for cisco and vice versa. Both species are, therefore, more susceptible to wide fluctuations in population abundance owing to changing climatic conditions (Shuter and Post 1990; Carpenter et al. 1992). Long-term analyses of climate records for the Great Lakes basin (Johnson and Evans 1990; Robertson et al. 1992) indicate a significant increase in summer temperatures in the 1930s with winter temperatures peaking in 1930 and again in 1950. These observations coincide with the cisco decline and the white bass population increase in Lake Mendota. Climatic extremes have less impact on yellow perch; their populations have been far less variable than those of the other two fishes.

During the past three decades, system productivity has become more variable in Lake Mendota (Hurley et al. 1992; Kitchell and Sanford 1992; Lathrop 1992). Large die-offs of white bass and cisco in 1976 and 1987 have straddled strong recruitment by the same species in 1991 and 1977. Yellow perch recruitment has been extremely variable during this period. Extreme nutrient loadings associated with heavy precipitation in 1993 (Soranno 1995) and variable piscivore stocking and exploitation have also contributed to the heightened instability in the Lake Mendota food web. Because increased urban growth and changing land use practices can be anticipated (Soranno et al. 1996), those coupled with stochastic recruitment and inevitable climatic events suggest that this instability will probably continue for years to come. Effective constraint of the effects of urban growth and production of allochthanous nutrient loading will be essential if increased instability is to be prevented.

Evaluating the potential of biomanipulation as a management tool for improving water quality in Lake Mendota leads to an important general conclusion. Cisco are far more efficient zooplanktivores than yellow perch or white bass, and their abundance is critical in determining whether *D. pulicaria* will dominate. Further, cisco quickly outgrow their vulnerability as prey for all but the largest piscivores. Newly recruited YOY cisco would be an ideal prey for stocked piscivores (walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*)) owing to their fusiform morphology and lack of rigid spines; however, spatial overlap between cisco and potential predators is low (Johnson et al. 1992). Of the three zooplanktivores, individual yellow perch consume the least amount of *D. pulicaria* in their lifetime, yet they are the most susceptible to piscine predation (Johnson et al. 1992). While small white bass could constitute a large proportion of piscivore diets, increased predation on their populations will do little to restrict high spring biomass of *D. pulicaria*. Continued improvements in the water quality of Lake Mendota through biomanipulation will require sustained low cisco biomass. Unfortunately, the cisco population is only modestly dependent on the current fisheries management practices, which foster piscivore populations. Further reductions in nutrient loading and ecologically responsible land use planning (Soranno 1995) are the prerequisites of improved water quality or, at the least, reduction in the rate of water quality degradation.

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Effect of temperature and salinity on development and survival of eggs and free-living larvae of sealworm (*Pseudoterranova decipiens*)

Lena N. Measures

Abstract: Eggs of sealworm (*Pseudoterranova decipiens*) were subjected to 12 treatments involving four temperatures (0, 5, 10, 15°C) and three salinities (fresh water, brackish water, seawater). Eggs developed and larvae hatched in all treatments except at 0°C. Larvae hatched in 57 days at 5°C, 21–24 days at 10°C, and 10–11 days at 15°C. Mean percent hatch of larvae was 95–99% in treatments at 5, 10, and 15°C. After 1 year at 0°C, sealworm eggs had not hatched, and when placed at 18–20°C, very few larvae developed and hatched (0–6%). In brackish water or seawater, hatched larvae survived 91 days at 5°C, 63–67 days at 10°C, and 43–44 days at 15°C. In fresh water, larvae survived 11, 7–8, and 4–5 days at 5, 10, and 15°C, respectively. These data suggest that the low water temperatures recently observed off the east coast of Canada may influence transmission of sealworm to first intermediate hosts, their development in these hosts and subsequent transmission, and distribution in fish.

Résumé : Le développement des oeufs de ver du phoque (*Pseudoterranova decipiens*) a été observé dans 12 conditions différentes impliquant quatre températures (0, 5, 10 et 15°C) et trois salinités (eau douce, eau saumâtre, eau de mer). Les oeufs se sont développés jusqu'à l'éclosion des larves dans toutes les conditions sauf à 0°C. Les larves ont éclos après 57 jours à 5°C, 21–24 jours à 10°C et 10–11 jours à 15°C. Le pourcentage moyen d'éclosion des larves varie de 95–99% à 5, 10 et 15°C. Après un an à 0°C, les oeufs de ver du phoque n'étaient pas encore éclos. Lorsqu'ils ont ensuite été placés à 18–20°C, très peu de larves se sont développées et ont éclos (0–6%). En eau saumâtre et en eau de mer les larves écloses ont survécu 91 jours à 5°C, 63–67 jours à 10°C et 43–44 jours à 15°C. En eau douce, les larves ont survécu 11, 7–8 et 4–5 jours à 5, 10 et 15°C, respectivement. Ces données suggèrent que les basses températures de l'eau observées récemment au large de la côte est du Canada peuvent avoir un effet sur la transmission du ver du phoque aux premiers hôtes intermédiaires et sur son développement à l'intérieur de ces hôtes. La transmission subséquente aux poissons et la distribution du parasite pourraient aussi être influencées.

Introduction

Development of early free-living stages of sealworm (*Pseudoterranova decipiens*) has been extensively studied. Effects of different temperature regimes on developing eggs of sealworm were studied by Scott (1955), Myers (1960), McClelland (1982), Brattey (1990), and Burt et al. (1990). Survival of sealworm larvae post-hatch at different temperatures was also investigated by McClelland (1982) and Burt et al. (1990). Effects of different salinities at different temperature regimes on developing sealworm eggs and survival of larvae post-hatch were studied by Burt et al. (1990).

During the early 1990s, some waters off the east coast of Canada were particularly cold, with intense negative temperature anomalies being recorded (Colborne and Fitzpatrick 1994). The development and survival of free-living stages, especially in suboptimal conditions, is of particular interest to parasitologists in fisheries research who wish to understand the distribution of sealworm and the dynamics of its transmission to various hosts including being able to predict changes that may occur as environments change. The purpose of the present paper is to report results of experiments conducted with eggs and larvae of sealworm subjected to a range of temperatures, including O°C, and salinities for up to 1 year. Data on time for larvae to hatch, mean percent hatch, and survival time of larvae post-hatch at four temperatures and at three different salinities are given. Previous studies have not investigated the effect of prolonged cold water temperatures (O°C) at different salinities on development and survival of eggs and larvae of sealworm.

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Materials and methods

Sealworm were obtained from adult grey seals (*Halichoerus grypus*) collected by shooting with permission in the Northumberland Strait, Gulf of St. Lawrence, near Port Hood (46°01′N, 61°32′W) and Merigomish (45°38′N, 62°26′W), Nova Scotia, Canada, during January. Gravid sealworm removed from the stomach of grey seals were washed twice in mammalian saline and kept in saline at 0°C. In the laboratory the distal extremities of uteri were removed from four gravid females. Eggs that were in the early morula stage were dissected from uteri and up to a few thousand eggs were placed in each of 75 covered Petri dishes (35 × 10 mm) containing either fresh water ($S = 0$), brackish water ($S = 17$), or seawater ($S = 35$). Salt water was made using synthetic sea salts (Tropic Marin, Dr. Biener GMBH Aquarientechnik, 6423 Wartenberg, Germany) and deionized water. Salt concentrations were verified using a hydrometer.

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Table 1. Time (days) for larval sealworm (*Pseudoterranova decipiens*) to hatch from eggs kept at various salinities and temperatures.

	Salinity		
Temperature (°C)	Seawater	Brackish water	Fresh water
15	11	10	10–11
10	22–24	21–22	21–22
5	57	57	57
0	—	—	—

Table 2. Percent hatch of larval sealworm (*Pseudoterranova decipiens*) from eggs kept at various salinities and temperatures.

	Salinity		
Temperature (°C)	Seawater	Brackish water	Fresh water
15	96.7±1.3	99.3±0.4	95.4±1.1
10	98.7±0.2	98.2±0.9	95.6±1.2
5	98.0±0.8	98.0±1.1	94.8±0.6
0[a]	0	0	0

Note: Values are given as the mean ± SD.
[a]Eggs were kept at this temperature for 1 year.

Table 3. Survival time (days) of larval sealworm (*Pseudoterranova decipiens*) kept at various salinities and temperatures post-hatch.

	Salinity		
Temperature (°C)	Sea water	Brackish water	Fresh water
15	43	44	4–5
10	65–67	63–64	7–8
5	91	91	11
0	—	—	—

Five replicates of each salinity treatment (fresh water, brackish water, seawater) were placed in incubators (Fisher Scientific low-temperature incubator, model 307) at each temperature treatment (0, 5, 10, and 15°C) (Table 1). Photoperiod was not controlled. Temperatures were monitored daily. Daily monitoring of temperatures at which eggs were incubated provided the following values (mean ± SD): –0.1 ± 0.2, 4.7 ± 0.4, 10.6 ± 1.0, and 15 ± 0.5°C. Solutions were agitated daily or once per week and replaced at intervals as needed.

Development, time of hatch, total number of eggs, total number of hatched larvae, and survival time of hatched larvae in each treatment were monitored daily or weekly depending on development and larval activity, using an inverted microscope. Survival time of hatched larvae was determined as the period from hatching until movement ceased and the majority of larvae were considered dead and degenerating. The means of the log of (hatched larvae + 1)/(total number of fertilized eggs + 1) were analyzed for each treatment (two-factor analysis of variance). The least significant difference (LSD) test was used to identify where differences occurred.

Results

Sealworm larvae hatched in all treatments except those at 0°C (Table 1). Development to hatching was most rapid at 15°C, with all larvae hatching within 10–11 days in the three salinities. Generally, larvae hatched up to 1 day faster and in greater percentages in brackish and fresh water than in seawater.

Mean percent hatch of sealworm larvae did not differ between treatments at 15, 10, or 5°C ($P > 0.05$) (Table 2). At 0°C, larvae did not hatch; after 1 year at 0°C sealworm eggs had not developed beyond the early tadpole stage. These eggs were then placed at room temperature (18–20°C); after 15 days from 51.4 to 95.5% of eggs became larvated, but none hatched in seawater, only 3.0 ± 4.0% hatched in brackish water, and 5.8 ± 2.2% hatched in fresh water. No movement was observed in the remaining larvated eggs, which appeared dead and degenerating.

The optimal temperature and salinity for survival of larval sealworm post-hatch was 5°C in either brackish water or seawater (Table 3). Although larvae developed and hatched in fresh water, post-hatch survival was of short duration (4–11 days) depending on temperature.

Discussion

Data presented here indicate that, while exposure to a temperature of 0°C for 1 year does not kill sealworm eggs nor prevent them from resuming development to the larval stage when temperatures are increased, a prolonged period of cold appears to affect their ability to complete development and hatch at 18–20°C (only 3% hatched in brackish water). McClelland (1982) reported that most sealworm eggs remained at the 2- to 8-cell stage at 0–1°C in seawater for 150 days after which they were then placed at 15°C where only 58% of larvae developed and hatched. In the present study, some eggs were in the early tadpole stage after 117 days at 0°C, but these eggs, which were retained at 0°C for longer than any previous studies, did not develop further after 1 year at 0°C. Incubators were closely monitored daily and for the 0°C treatment group temperatures fluctuated between –0.5 and 0.3°C (mean –0.1°C) during the 1-year course of the experiment. Brattey (1990) kept sealworm eggs at –0.5°C in seawater for 1–3 months after which they were placed at 12°C where 72–96% hatched. Inability to hatch after exposure to low temperatures for long periods may be due to diminished larval energy reserves needed to leave the egg.

Time of sealworm larvae to hatch at 15, 10, and 5°C in seawater was similar to that reported by McClelland (1982), Brattey (1990), and Burt et al. (1990). The present observations that sealworm larvae hatched slightly faster in fresh water and brackish water were also observed by Burt et al. (1990). The slight temporal differences betwen studies can probably be attributed to osmotic conditions that facilitate the escape of larvae from eggs as the permeability of the egg shell changes prior to hatch (see Bird and Bird 1991, p. 35).

Mean percent hatch of larval sealworm was higher in the present study (95–99%) than that (80–95%) reported by Brattey (1990). These differences may be due to different experimental design. For example, in the present study only sealworm eggs removed from the distal extremity of uteri and enclosed within a shell were used for experiments. Burt et al. (1990) indicated that not all sealworm eggs used in their experiments were fertilized and that only about 85% hatched. McClelland (1982), using an experimental design similar to that of Brattey (1990), observed sealworm prehatch mortalities of 3–11% at 5–7°C, 9–28% at 10–17°C, and 22–38% at 20°C.

McClelland (1982) observed that sealworm larvae survived 140 days post-hatch in seawater at 5°C but only 6 days at 15°C. This is markedly different from the present study where larvae survived only 91 days in seawater or brackish water at 5°C but longer (43 days) at 15°C. Burt et al. (1990) also reported

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shorter survival times for sealworm larvae post-hatch in seawater or brackish water at 11–12°C and at 15°C (maximal survival time at 4°C was not determined, although the larvae were still active after 60 days) compared with the present study. As nematode larval survival is also influenced by oxygen availability, the above differences may be accounted for by how often solutions were agitated or replaced with fresh solutions at the same temperature during the course of studies.

This study and that of Burt et al. (1990) indicated that survival times of larvae post-hatch in fresh water were very brief (3–5 days) but that larvae survived equally well in seawater and brackish water. This ability to survive a range of salinities, such as may be found commonly in coastal estuaries, is demonstrated by the occurrence of sealworm in various species of fish such as American plaice (*Hippoglossoides platessoides*) that inhabit the lower estuary of the St. Lawrence River (L.N. Measures, unpublished data), which seals frequent (Boulva and McLaren 1979; Sergeant 1991; Lavigueur et al. 1993).

Transmission studies have indicated that sealworm eggs and larvae (L1, L2, L3) are particularly sensitive to low temperatures (see Ronald 1960; McClelland 1982; Burt et al. 1990). For example, copepods did not become infected when exposed to larval sealworm stored at 0 or 5°C for more than 120 days (McClelland 1982). Infectivity of sealworm larvae retained at 0°C for various periods (to invertebrate hosts) was not determined in the present study. Infectivity experiments with sealworm larvae and invertebrate hosts cultured at low temperatures, such as 0°C, need to be conducted to determine whether larvae that develop at 0°C or are retained at 0°C for up to 1 year are still infective to various invertebrates.

Temperature is the most important abiotic factor influencing the development and transmission of parasites (see Overstreet 1982; Williams and Jones 1994). Brattey (1990) examined water temperatures recorded off the east coast of Canada and determined that sealworm would require more than 4 months to hatch in Labrador offshore areas where monthly mean temperatures were low (–1.5 to 2°C). The present study indicates that few sealworm eggs would hatch where temperatures were consistently this low, thus reducing transmission to invertebrates. Transfer of larval sealworm (L3) from small migratory prey fish to large predatory fish may be inhibited at low temperatures, as Ronald's (1960) data suggest, thus further reducing the overall abundance of sealworm in fish consumed by seals. This may explain the reported low prevalence and abundance of sealworm in northern cod (*Gadus morhua*) and American plaice stocks off Labrador and northeastern Newfoundland (see Templeman et al. 1957; Brattey et al. 1990).

On the east coast of Canada, as reported by Brattey (1990), offshore areas showed little or no seasonal temperature cycle whereas most inshore areas experienced seasonal changes, with temperatures usually below 0°C from January to April, after which temperatures increased, reaching maximal values in July or August to October or November depending on location. As shown by the present and previous studies, development of sealworm eggs accelerates as temperatures increase; however, survival and infectivity of hatched infective larvae diminish as temperatures increase. Consequently, infective larvae ingested by suitable invertebrate hosts as soon as possible are more likely to be transmitted to subsequent hosts than those remaining longer in the external environment. It follows that the probability of transmission is increased when these hosts are abundant and at a susceptible age or size. McClelland (1990) showed that larval amphipods (<5.0 mm) are more susceptible to infection than adult amphipods (≥5.1 mm) of the same species. Sealworm larvae develop more rapidly in invertebrates held at warm temperatures (15°C) than in those held at cold temperatures (5°C) (McClelland 1982, 1990), and only sealworm larvae ≥1.41 mm in length are infective to fish (McClelland 1995). Thus, larvae infecting young susceptible invertebrates and developing to this size (20 days at 15°C, 50 days at 10°C, and 135 days at 5°C in amphipods according to McClelland 1990) have the greatest probability of being transmitted subsequently to fish. Optimal environmental temperatures with an abundance of young susceptible invertebrates frequently occur during summer to early fall in Canadian east coast inshore waters. To understand the dynamics of transmission relative to water temperatures, studies of seasonal patterns of infection in invertebrates in inshore areas would be instructive.

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Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeophtheirus salmonis* Krøyer): migration, growth, and mortality

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Abstract: Premature return of sea trout (*Salmo trutta*) was studied by collecting ascending trout in a fish trap in the mouth of the Lønningdalselven River, western Norway. Postsmolts ascended the river in June and older migrants in July. Ascending fish were heavily infested with salmon lice (*Lepeophtheirus salmonis*) and many were in poor physical condition. Louse infestations differed between the two groups of anadromous trout. Postsmolts had a median infestation intensity of 206 lice, mainly juveniles. Older migrants carried a median intensity of 43.5 lice, with a high proportion of preadult and adult lice. Forty-one percent of the postsmolts that ascended the river migrated back to the sea, after a median river stay of 37.5 days. These fish had recovered from the severe louse attack but experienced a median decrease of 23.5% in body mass and no increase in length. All older migrants stayed in the river for the rest of the season. Low mortality (3.6%) was observed in prematurely ascending postsmolts but 19.7% of the older migrants died within the 1st week of their return.

Résumé : Nous avons étudié le retour précoce de la truite de mer (*Salmo trutta*) en prélevant des poissons en remonte dans un piège installé à l'embouchure de la rivière Lønningdalselven, dans l'ouest de la Norvège. Les postsmolts remontaient la rivière en juin et les poissons plus âgés en juillet. Les poissons en remonte étaient gravement infestés de poux du saumon (*Lepeophtheirus salmonis*), et bon nombre d'entre eux étaient en piètre condition physique. Les infestations différaient d'un groupe à l'autre parmi les truites anadromes. Les postsmolts présentaient une intensité médiane d'infestation de 206 poux, surtout juvéniles. Les migrants plus âgés présentaient une intensité médiane de 43,5 poux, dont une forte proportion de préadultes et d'adultes. Parmi les postsmolts en remonte, 41% sont redescendus en mer, après un séjour médian en rivière de 37,5 jours. Ces poissons s'étaient remis de la forte attaque des parasites, mais ils présentaient une baisse médiane de 23,5% de la masse corporelle et aucune augmentation de la longueur. Tous les migrants plus âgés sont restés dans la rivière pour le reste de la saison. Une faible mortalité (3,6%) a été observée chez les postsmolts en remonte précoce, mais 19,7% des migrants plus âgés sont morts dans la semaine qui a suivi leur retour.
[Traduit par la Rédaction]

Introduction

Sea trout smolts (*Salmo trutta* L.) leave the rivers in spring and migrate to coastal areas where they feed during summer (Jensen 1968; Jonsson 1985). The time spent and conditions in the sea may have a major impact on sea trout development, affecting time of return and reproductive success of these fish. Some migrants spend the whole 1st year in the sea and do not return to the river before they mature. Most sea trout, however, return to fresh water in the autumn and overwinter in fresh water before reentering the sea the following spring (Nordeng 1977). This annual pattern is repeated for the rest of their lives.

Several factors are known to be related to time spent at sea. Average residence time in the sea decreases with increasing latitude (L'Abée-Lund et al. 1989). Sea trout from Vardnes River, northern Norway, returned within an average of 68 days (Berg and Berg 1989), while sea trout from River Vosso, southern Norway, spent on average more than 90 days in the sea before returning (Jonsson 1985; Jonsson and Gravem 1985). Residence time in the sea also depends on number of seaward migrations, and postsmolts generally return later in the season than older migrants (Nordeng 1977; Jonsson 1985). In recent years, however, sea trout postsmolts have been observed ascending Irish estuaries and rivers within a few weeks after their smolt migration (Tully et al. 1993*a*, 1993*b*), a phenomenon called premature return. These early ascending postsmolts are usually heavily infested with salmon lice (*Lepeophtheirus salmonis* Krøyer) (Tully et al. 1993*a*, 1993*b*) and have grown little during their short seawater stay (Tully et al. 1993*a*). An association between louse infestation and premature return has been suggested (Tully et al. 1993*a*; Birkeland and Jakobsen 1997). If such a relation is true the lack of growth may result from the energetic cost of louse infestations, the short time spent in seawater, or both.

Feeding by lice causes erosions and sores in the fish's skin (Wootten et al. 1982; Jònsdòttir et al. 1992), leading to osmoregulatory problems in salt water (Grimnes and Jakobsen 1996). Migration to estuarine water may enable a fish to retain its osmotic balance, and hence survive. Further, the louse has a low tolerance to low salinity and will die if its host stays in brackish or fresh water (Hutton 1923; Berger 1970; Hanenkamp and Fyhn 1985). Generally, louse-induced pathology

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Table 1. Mean total length (cm), wet mass (g), and condition factor for sea trout ascending the Lønningdalselven River, June–July 1992.

	N	Length	Mass	Condition factor
Postsmolts	94	17.4±2.1	42.3±15.5	0.78±0.09
Older migrants	78	37.4±7.3	580.7±346.8	0.99±0.14

Note: Values are given as the mean ± SD.

increases with both number and development of lice, and severe skin damage to the host is caused mainly by preadult and adult lice (Grimnes and Jakobsen 1996).

Although premature return of louse-infested sea trout has been observed in many rivers, there have been few studies on the direct consequences of early river return. The aim of the present study was therefore to examine the effect of louse infestations on the returning sea trout, their migratory behaviour, and their growth and mortality in fresh water.

Methods

The study site

A fish trap with separate collection boxes for descending and ascending fish was constructed at the high-water mark of the Lønningdalselven River, in Lønningdal on the west coast of Norway. Ascending and descending fish had to pass through the trap, and the number of fish passing without being recorded was therefore considered negligible.

The Lønningdalselven River has a catchment area of 17.2 km^2, and from the river mouth in the Samnangerfjorden the fish can migrate 1 km upstream to Lake Øvredalsvatnet where populations of brown trout and Arctic char (*Salvelinus alpinus* L.) are found. From the northern side of the lake fish can migrate freely about 2 km up the Øvredalselven River.

Descending fish were caught from late April 1992 and the ascending fish from early June 1992. Until September the fish trap was examined twice a day for migrating fish whereas in September the trap was removed because of high water flow.

Sea trout migration, growth, and mortality

Migrating sea trout were classified as postsmolts (first-time migrants) and older migrants (two or more seaward migrations) according to their size (Table 1), and there was no overlap in size between fish in the two groups. Sea trout collected in the trap were anaesthetized with metomidate hydrochloride (5 mg/L) and their wet mass (±1.0 g) and total length (±0.1 cm) recorded. The condition factor (CF) was calculated according to the formula

$$CF = W \times 100 \times TL^{-3}$$

where W is wet mass (in grams) and TL is total length (in centimetres).

The specific growth rate (SGR) of postsmolts while in the river, expressed as percentage per day, was calculated according to the formula

$$SGR = (\ln W_t - \ln W_0)100 \times t^{-1}$$

where W_0 is the fish wet mass when ascending the river (day 0), W_t is the mass when leaving the river, and t is the number of days spent in the river.

All sea trout ascending the Lønningdalselven River were individually marked with visible implant tags (VI tags) (Anonymous 1992) behind both eyes, and their adipose fin clipped. Smolts leaving the Lønningdalselven River from late April until June 1 were collected and used in another experiment (Birkeland and Jakobsen 1997). Consequently, sea trout ascending the Lønningdalselven River in this study must have left the river before the descending fish trap was built, stayed the winter at sea, or originated from other rivers. Because of the latter possibility, unless referring directly to the phenomenon of premature return, I use the term ascending instead of the term return in this study.

Numbers of lice were counted and louse stages recorded according to Johnson and Albright (1991) as copepodid, chalimus, preadult, adult male, and adult female without or with egg strings. Ascending fish were released upstream of the trap and descending fish downstream of the trap. Every 10th ascending fish was frozen so that it could be examined in the laboratory later. The chalimus stages were then separated into chalimus I and II (combined) or chalimus III and IV (combined). The preadult I and preadult II stages were separated, and females distinguished from males. However, low numbers of laboratory-examined fish necessitated combining data with data from field-examined fish.

The estuary and the lower parts of the river were searched daily for dead and moribund sea trout (fish swimming slowly in the water surface or hyperventilating on the bottom of the estuary). The estuarine area was small with a water level less than 1 m. Moreover, the bottom was sand covered and the vegetation sparse so fish could easily be observed through the water.

Statistical analysis

Infestation data were not normally distributed (Kolmogorov–Smirnov test, $p < 0.001$), and the variance differed among the groups (Levene test for homogeneity of variances, $p < 0.001$). Transformations did not fulfill the requirements for parametric statistics so nonparametric statistics were mainly used in this study. Consistent with the use of nonparametric tests, infestation parameters are presented as median values with interquartile range (IQR). The Wilcoxon Mann–Whitney test was used for comparisons between two samples and the Kruskal–Wallis test for comparisons among more than two samples (comparisons of louse stages within postsmolts and older migrants). If the p value in the Kruskal–Wallis test was less than 0.05, a multiple comparison between treatments for the Kruskal–Wallis test (Siegel and Castellan 1988) was employed as a post-hoc test. The Kolmogorov–Smirnov two-sample test was used to compare the louse developmental stages between two groups, and the Spearman rank correlation was used for correlation analyses. Tests were carried out using an SPSS computer program. Parasitological terms follow Margolis et al. (1982).

Results

The first ascending postsmolts were observed in the estuary in early June, and during the 3 following weeks ascending sea trout postsmolts were collected almost daily from the fish trap. However, from the end of June until mid-July only a few fish were caught. Upstream migration started again in mid-July, lasting for 2 weeks. The last ascending fish were mainly older fish that had migrated to and from the sea for several years. Within each of the two groups of sea trout no correlation was found between the time of ascent and the number of lice (Spearman rank correlation, $r_s = 0.05$ (for both postsmolts and older migrants), $p > 0.6$) but postsmolts ascended with higher numbers of lice than older migrants (Wilcoxon Mann–Whitney, $Z = -6.38$, $p < 0.01$) (Table 2). Moreover, developmental stages of lice differed between the postsmolts and the older migrants (Kolmogorov–Smirnov two-sample test, $D_{[7004,\ 24\ 518]} = 0.22$, $p < 0.001$) (Fig. 1). Postsmolts were mainly infested by copepodids and chalimus larvae (multiple comparisons between treatments, $p < 0.05$). Older migrants also carried preadult and adult lice in high numbers, and adult lice dominated compared with preadults (multiple comparisons between treatments, $p < 0.05$) (Fig. 1).

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Table 2. Infestation parameters for salmon lice, parasitic on sea trout returning to the Lønningdalselven River, June–July 1992.

		Prevalence	Abundance		Intensity			
	N	(%)	Mean	SD	Mean	Median	IQR	Max.
Postsmolts	94	96	260.8	218.1	272.4	206.0	254	1002
Older migrants	78	87	89.8	132.7	103.0	43.5	115	522

Note: *N*, number of fish examined; IQR, interquartile range; max., maximum number of lice found on a single fish. Parasitological terms follow Margolis et al. (1982). Prevalence is the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined. Intensity is the number of individuals of a particular parasite species in each infected host in a sample. Abundance is the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species (infected and uninfected) in the sample.

Fig. 1. Developmental stages of the salmon louse on sea trout postsmolts (open bars, $n = 24\,515$) and older migrants (solid bars, $n = 7004$) ascending the Lønningdalselven River in June and July 1992.

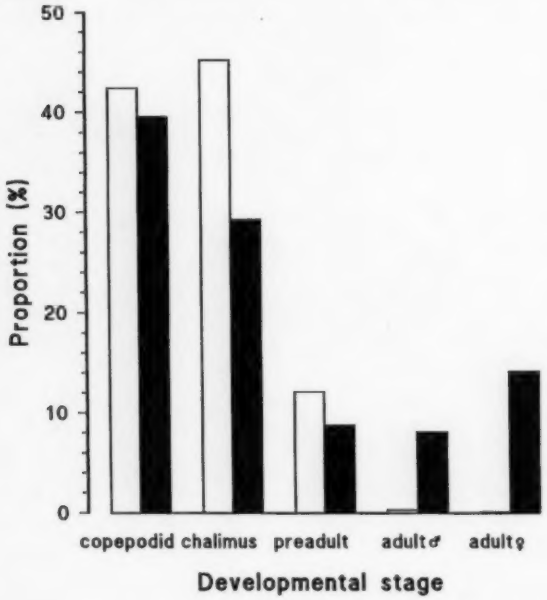
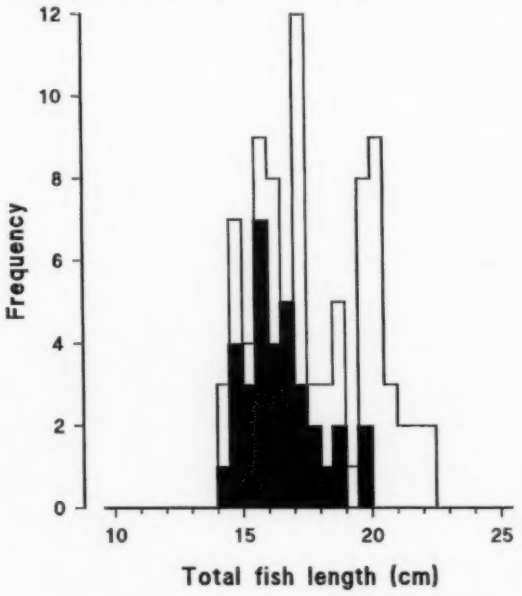


Fig. 2. Length distributions of 94 sea trout postsmolts ascending the Lønningdalselven River in June 1992 (open bars) and those 34 descending the river again in the same season (solid bars).



All older migrants that ascended to the trap remained in the river during the monitoring period. However, 34 of the 83 (41.0%) postsmolts that were released upstream of the trap migrated back to sea. These fish had stayed a median of 37.5 days in fresh water (range 7–46 days) before leaving the river, but no correlation was found between louse numbers when ascending and the number of days spent in the river (Spearman rank correlation, $r_s = 0.05$, $p > 0.7$). Further, neither louse numbers (Wilcoxon Mann–Whitney, $Z = -0.14$, $p > 0.8$) nor louse stages (Kolmogorov–Smirnov two-sample test, $D_{[13\,764,\,7823]} = 0.01, p > 0.05$) differed between postsmolts that reentered the sea and postsmolts that were not observed reentering the sea. However, those postsmolts that descended the river again were smaller (Fig. 2) and weighed less during ascent than postsmolts that remained in the river during the study period (t test, $t = 2.48$ (length), $p < 0.02$; $t = 2.33$ (mass), $p < 0.03$). Their condition factors, however, did not differ (t test, $t = -1.26$, $p > 0.2$).

Thirty postsmolts (88.2%) lost mass during the freshwater stay. The specific growth rate of mass (SGR) was negative with a median of –0.9%/day (range +1.1 to –3.5%) and during the freshwater stay the 34 postsmolts lost a median of 23.5% (range +22.3 to –37.1%) of their body mass. However, no correlation between number of days spent in the river and mass loss was found (Spearman rank correlation, $r_s = 0.30, p < 0.2$). Moreover, the descending fish did not increase in length during their stay in fresh water, nor was there any correlation between louse infestations on ascending postsmolts and mass loss (Spearman rank correlation, $r_s = -0.19, p < 0.4$).

When the postsmolts descended, most fish had lost all of their lice. Lice were only found on three descending fish, but in a much lower number than when ascending. These fish had stayed from 10 to 16 days in fresh water and carried 1–21 lice. Most of these lice were chalimus larvae, but five preadult lice were observed on one fish. Most likely the chalimus had been attached to the fish since they returned to fresh water whereas preadults could have transferred (Bruno and Stone 1990) from ascending to descending fish in the river.

Only three ascending postsmolts were found dead in the

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river and estuary. However, high freshwater mortality was recorded among older migrants. Within the 1st week after their freshwater ascent 14 of the 71 (19.7%) older migrants that were released upstream were found dead in the lower parts of the river. When ascending, these fish had considerable mucus and skin lesions, probably caused by the salmon louse, and in fresh water they soon became infected by fungus. However, the number of lice did not differ between older migrants that died and those that were not found dead and thus may have survived (Wilcoxon Mann–Whitney, $Z = -0.02$, $p > 0.9$).

Discussion

In the Lønningdalselven River in western Norway, sea trout postsmolts returned prematurely to the river whereas older migrants ascended closer to the normal time of their return. Both postsmolts and older migrants were heavily infested by salmon lice and were weakened as a result. At least 20% of the older migrants died within their 1st week in fresh water but only three postsmolts were observed dead. Of the postsmolts that ascended prematurely, 41% migrated back to sea. These fish had stayed more than a month in the river and had lost approximately one quarter of their mass. None of the older migrants returned to sea during the study period.

Prematurely returning sea trout have been observed in recent years both in Ireland (Tully et al. 1993*a*, 1993*b*) and in Norway (K. Birkeland, personal observation). Generally it is the postsmolts that return prematurely, while premature return of older migrants is not commonly reported (Tully et al. 1993*a*, 1993*b*). This seems to be in accordance with the results from the Lønningdalselven River. In western Norway, freshwater migration generally starts in July–August, and older migrants normally return earlier than younger migrants (Jonsson 1985). Hence, while postsmolts returned prematurely, it is questionable whether older migrants did so.

Growth in the sea is generally better than in fresh water (Jonsson 1985; L'Abée-Lund et al. 1989; Jonsson and L'Abée-Lund 1993; Frier 1994). Premature return may therefore affect growth of sea trout. In this study 41% of the postsmolts returned to the sea in the same season. Those postsmolts that descended the river again were smaller and weighed less during ascent than postsmolts that remained in the river during the study period. It is therefore possible that their seaward migration is size dependent. However, although some postsmolts reenter the sea, they may have lost 30% of their potential sea growth for that year, while the postsmolts that remained in fresh water lost the rest of their sea growth. Freshwater residency may have severe effects on fish growth, as shown by the postsmolts that returned to the sea. During their freshwater stay postsmolts lost body mass and did not grow in length. The postsmolts ascending the Lønningdalselven River had severe skin damage and probably had high energetic costs of osmoregulation and repair of damaged tissue. In addition, reduced feeding in fresh water may cause further decreases in body mass. However, no correlation was found between length of river stay and mass loss, although variations in stress, tissue damage, and fat depositions may have masked such an association. Moreover, sea trout may have started to feed after a given period in the river, thus preventing continued mass loss.

Of the sea trout that returned to the study river more than 90% were heavily infested with lice, and postsmolts carried a median intensity of more than 200 lice. By far the most frequent louse stages on the returning postsmolts were the chalimus stages. A similar louse demography was recorded on returning postsmolts in Ireland (Tully et al. 1993*a*, 1993*b*). A finding in contrast to other studies (Tully et al. 1993*a*, 1993*b*; K. Urdal, Institute of Zoology, University of Bergen, personal communication) was the high proportion of copepodids (the infective stage) on ascending fish, which may be due to a substantial infection pressure in the area. However, sea trout were sampled twice a day in this study, while there were several days between samplings in the other studies. The limited exposure of the fish to fresh water before they were examined may therefore have meant that stages of lice were recorded that were lost by the time fish were examined in other studies. Moreover, since all louse stages were frequently observed on ascending fish throughout the observation period, salmon louse infestation may be a continuous process and not a discrete one, as suggested by Tully et al. (1993*a*).

Salmon lice may influence their hosts severely, and as few as five adult lice have been reported to cause significant pathology on Atlantic salmon (*Salmo salar* L.) smolts (Wootten et al. 1982). Host damage generally increases with both the number and stage of lice. Juvenile stages of lice seem to cause little damage to the host (Jones et al. 1990), and in an experimental study with relatively high infestations (median of 64 lice and a range of 30–250 lice) no osmotic stress was noted in Atlantic salmon postsmolts before lice reached the preadult stage (Grimnes and Jakobsen 1996). However, immediately after the lice moulted to preadults the fish developed osmoregulatory problems and started to die. Less than 30 preadult lice caused mortal damage to postsmolts, and all postsmolts died before the appearance of the adult louse stage (Grimnes and Jakobsen 1996). A similar mortality pattern was observed in sea trout postsmolts (Birkeland and Jakobsen 1997).

In this study older migrants seemed to suffer a higher freshwater mortality than the postsmolts, even though the infestation density (number of lice per fish mass) (Margolis et al. 1982) was 25 times higher on postsmolts than on the older migrants. However, older migrants carried a higher proportion of older louse stages, which may have caused a higher mortality rate. No systematic registration of fish mortality was performed, although daily observations were made in the estuary and in the lower parts of the river, and ascending fish could therefore have died in other parts of the river or been removed by predators. Mortality may consequently have been higher than observed, particularly for the postsmolts because of their small size.

Owing to the sudden increase in fish mortality with the preadult louse stages found by Grimens and Jakobsen (1996) it may be important for the fish to delouse before preadult lice appear. This suggests that heavily infested sea trout return to fresh water before too many lice develop into older and detrimental stages. That most ascending sea trout are infested with high numbers of salmon lice, of which juvenile stages are the most frequent (Tully et al. 1993*a*, 1993*b*; the present study), supports this hypothesis. The benefit of freshwater return is, however, difficult to evaluate, since comparative data from fresh water and from the sea are not available. Firstly, although there is much information about louse infestations on sea trout

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returning to fresh water, there is only one observation of louse numbers and stage distribution on sea trout collected in marine coastal areas (Boxshall 1974). It is therefore unknown whether heavily infested sea trout ascend estuaries and rivers while less infested fish remain in coastal areas. However, unpublished data on sea trout postsmolts collected simultaneously from marine and estuarine locations in Hardangerfjorden, western Norway, in May 1992 showed that the marine fish carried fewer lice and younger louse stages than sea trout postsmolts collected from estuaries close by (K. Birkeland and A. Grimnes, unpublished data). However, the sea trout in Hardangerfjorden were collected some weeks earlier in the year and were less heavily infested than the sea trout ascending the Lønningsdalselven River. Louse demography on the marine and the estuarine trout in Hardangerfjorden could therefore have changed at the time postsmolts ascended the Lønningdalselven River.

Secondly, equivalent mortality data for sea trout in fresh water and seawater are not available, and little is known about the mortality of lice on wild salmonids. In an experimental study with Atlantic salmon, Grimnes and Jakobsen (1996) recorded louse mortality of only 43% from early chalimus larvae to the second preadult stage. In the Lønningdalselven River returning postsmolts were infested with a median intensity of 90 chalimus larvae. If these sea trout had remained in the sea where lice could have developed into older stages, the results from the study by Grimnes and Jakobsen indicate that most ascending sea trout would have been infested with a lethal number of lice. By returning to fresh water, however, at least 40% of the ascending fish deloused, recovered, and redescended to the sea at a cost only of a lower growth rate.

Fecundity increases with body size (Jonsson 1985; L'Abée-Lund and Hindar 1990), and L'Abée-Lund (1994) found that sea age at first maturity was significantly influenced by growth rate at sea. Therefore, given that sea trout experience a high mortality rate because of salmon louse infestations, sea trout have to choose between the benefits of growth in the sea and the costs associated with a mortal impact from the louse. Unless the fish experience compensatory growth (Dobson and Holmes 1984; Broekhuizen et al. 1994) when they return to sea, their growth must be reduced compared with that of fish spending the whole growth season in the sea. Therefore, loss of mass and reduced growth as a result of louse infestation may reduce resources available for egg production, thereby reducing fecundity and reproductive success.

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Stable isotope evidence for dietary overlap between two planktivorous fishes in aquaculture ponds

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Abstract: There is ongoing debate on the feeding habits of silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*), which are widely cultivated fishes in fresh waters. Here we report stable carbon and nitrogen isotope values from five polyculture ponds in Zhanjiang, China, to determine carbon sources and trophic interaction between the two species. The $\delta^{13}C$ of phytoplankton, zooplankton, muscle tissue, and gut contents differed by 0.5–2.2‰ within each fishpond, indicating that the fish growth was supported by phytoplankton production. Progressive increases in $\delta^{15}N$ from phytoplankton, zooplankton, to fish were indicated. Mean muscle $\delta^{15}N$ values of bighead were 0.4 (pond V) to 2.3‰ (pond I) higher than those for silver carp. These isotopic differences were less than the 3–4‰ expected from feeding on two adjacent trophic levels and suggested that there were various degrees of dietary overlap between the two species among the fishponds. We estimated that, on the basis of an isotope model, silver carp and bighead obtained from 23 to 87%, with an average of 60%, of their food from the same trophic level.

Résumé : Les habitudes alimentaires de la carpe argentée (*Hypophthalmichthys molitrix*) et de la carpe marbrée (*Aristichthys nobilis*), espèces couramment élevées dans de nombreuses régions d'eaux douces, font depuis longtemps l'objet d'un débat. Nous rapportons ici des mesures d'isotopes stables du carbone et de l'azote dans cinq étangs de polyculture de Zhanjiang (Chine) afin de déterminer quelles sont les sources de carbone et l'interaction trophique entre les deux espèces. La teneur en $\delta^{13}C$ du phytoplancton, du zooplancton, du tissu musculaire et du contenu de l'intestin des carpes variait de 0,5 à 2,2‰ dans chaque étang, ce qui indique que la croissance des poissons était soutenue par la production de phytoplancton. Nous avons observé des augmentations progressives de la teneur en $\delta^{15}N$ du phytoplancton, du zooplancton puis des poissons. La teneur moyenne en $\delta^{15}N$ du muscle chez la carpe marbrée était de 0,4 (étang V) à 2,3‰ (étang I) supérieure à celle observée chez la carpe argentée. Ces différences d'ordre isotopique étaient inférieures aux 3–4‰ attendues de la consommation des deux niveaux trophiques adjacents et permettent de penser qu'il existe divers degrés de chevauchement sur le plan alimentaire entre les deux espèces d'un étang à l'autre. Nous avons estimé, à partir d'un modèle isotopique, que la carpe argentée et la carpe marbrée tiraient entre 23 et 87%, avec une moyenne de 60%, de leur nourriture du même niveau trophique.
[Traduit par la Rédaction]

Introduction

Silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*) are important cultivated fish in the world. Their feeding habits have been the subject of numerous studies, mainly using gut content analyses. These studies suggest that silver carp and bighead are planktivorous, with silver carp largely feeding on phytoplankton and bighead on zooplankton (e.g., Nie and Chiang 1954; Cremer and Smitherman 1980). On the basis of these observations, efforts have been made to enhance silver carp production by increasing phytoplankton standing crop and by manipulating algal community structure (see reviews by He and Li 1975; He 1987). Attempts to control phytoplankton biomass using silver carp as algal filters have also been reported (Smith 1985; Starling 1993).

It has been recognized, however, that silver carp cannot digest many algal species (Nie and Chiang 1954; He and Li 1975; Bitterlich 1985; Herodek et al. 1989), and the digestibility of some other algae is sometimes poor (Bitterlich 1985). Experimental stocking of silver carp to control phytoplankton biomass in ponds and lakes has often failed to reduce primary production (Laws and Weisburd 1990). Other studies show that zooplankton can be an important dietary component for silver carp (Nie and Chiang 1954; Opuszynski 1981). Stocking silver carp often significantly reduces microzooplankton biomass (Burke et al. 1986; Opuszynski 1979; Starling 1993). Some studies reveal that a large portion of ingested food in silver carp is zooplankton (Kajak et al. 1977), which sometimes exceed phytoplankton in quantity (Spataru and Gophen 1985; Zhuo and Lin 1990). There is less uncertainty regarding the food sources of bighead, which are often found to feed largely on zooplankton (Nie and Chiang 1954; Wang 1988; Zhou and Lin 1990).

There are several major drawbacks to gut content analyses. First, dietary examinations display only what animals ingest at any given instant and yield little information on what the animals assimilate for their growth. Heavy consumption of phytoplankton

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Table 1. Pond depth, area, total length (TL), %C, and %N of silver carp (SC) and bighead (BH) from five fishponds in Zhanjiang, China.

	Pond No.				
	I	II	III	IV	V
Depth (cm)	150	210	220	120	130
Area (m^2)	2800	2240	2507	2330	2000
TL (cm)					
SC	27.2±3.6	28.6±4.1	29.3±2.4	28.9±1.4	28.3±1.3
BH	27.3±9.5	26.0±3.5	27.5±1.3	27.2±1.8	28.3±2.5
%C					
SC	46.9±1.0	47.8±0.3	46.6±2.3	44.7±0.9	45.2±3.0
BH	45.8±0.4	46.2±2.0	46.3±0.8	46.2±2.3	47.0±1.0
%N					
SC	14.1±0.4	13.7±0.9	13.8±0.5	13.4±0.8	14.0±0.7
BH	14.3±0.1	14.1±0.7	14.3±0.1	14.1±0.6	14.6±0.3

Note: Values for TL, %C, and %N are given as the mean ± SD.

by silver carp does not necessarily mean that the fish relies on primary producers as its major food source because of low digestibility of many algal species. Secondly, identification of dietary composition in the gut is sometimes problematic. Zooplankton in the foregut of silver carp are lysed rapidly and are often indistinguishable from detritus (Bitterlich and Gnaiger 1984). Lastly, gut content analyses provide no information on feeding history of fish unless a frequent sampling strategy is adopted, which is usually impractical.

Natural abundances of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) have been used to identify sources of organic matter and to define trophic structure in aquatic food webs (see reviews by Fry and Sherr 1984; Owens 1987; Peterson and Fry 1987; Wada et al. 1991). Isotopic fractionation of carbon and nitrogen takes place during nutrient assimilation by animals. Because organic matter of different origins often possesses distinct isotope compositions and fractionation of ^{13}C is small (DeNiro and Epstein 1978) during carbon metabolism by animals, carbon isotopes are now used to trace flows of organic matter from primary producers to consumers (Fry 1991). Nitrogen isotopes are more reliable predictors of trophic level because of the large ^{15}N enrichment from one trophic level to another (Owens 1987; Peterson and Fry 1987). Field investigations and laboratory experiments indicate that $\delta^{15}N$ increases approximately 3–4‰ during each trophic transfer (Minagawa and Wada 1984; Fry 1988). Furthermore, $\delta^{13}C$ and $\delta^{15}N$ record the feeding history of animals and give insight into the actual food incorporated into animal bodies. Stable isotope analysis can be a powerful tool in both identifying the source of organic matter that supports fish production and understanding trophic interactions of planktivorous fish in polyculture systems.

Despite the recent advances in stable isotope analysis in natural aquatic ecosystems, few studies using stable isotopes have addressed the feeding relationships of cultivated aquatic animals. Schroeder (1983*b*) showed that the mean $\delta^{13}C$ (–17‰) of tank-grown blue tilapia (*Oreochromis aureus*) was similar to that of supplied feed (–18‰) and different from that of algae (–22‰), indicating that tilapia depended on artificial feed. In contrast, Anderson et al. (1987), using an isotope mixing model, showed that supplied feed provided only 23 and 47% of the assimilated carbon in a prawn grow-out system with benthic biota supporting between 53% and 77% of the growth. Schroeder (1983*a*) indicated that the $\delta^{13}C$ values of silver carp resembled those of planktonic organic matter in polyculture ponds. Guo et al. (1987) reported that the $\delta^{13}C$ values of silver carp and bighead from a fishpond in China reflected an autochthonous carbon source. The isotope studies of silver carp by Schroeder (1983*b*) and Guo et al. (1987) provided no convincing evidence for the relative importance of phytoplankton and zooplankton in the growth of silver carp because they possessed similar $\delta^{13}C$ values.

Our objectives were to assess the food sources and trophic interaction between silver carp and bighead by analyzing stable isotope compositions of plankton, gut contents, and muscle tissue. The conservative nature of carbon isotopes and the progressive enrichments of nitrogen isotopes during trophic transfers can be used as indicators of carbon flows and trophic levels in aquaculture food webs.

Study site

Samples were collected from five polyculture ponds at a fish farm located in Zhanjiang (21°01′N, 110°19′E), southern China. These ponds have water depths ranging from 120 to 220 cm and surface areas from 2000 to 2800 m^2 (Table 1) and are used as rearing facilities for large fingerlings of silver carp and bighead. Small numbers of common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), mud carp (*Cirrhina molitorella*), mrigal (*Cirrhina mrigala*), and tilapia (*Oreochromis niloticus*) were also stocked in these ponds. To promote plankton production, the ponds were fertilized several times a year with inorganic chemical compounds enriched with N and P.

Materials and methods

Phytoplankton samples were obtained with a water sampler, filtered through a plankton net (mesh size 100 μm) to remove large zooplankton, and concentrated. Zooplankton samples were obtained by filtering pond water into a plankton net (mesh size 200 μm) and picked under a dissecting microscope. Three to five individuals of silver carp and bighead were randomly harvested from each fishpond. Dorsal muscle tissue (5 g) and diets from foregut were collected from each individual after total length (TL) was measured. All samples were dried at 70°C in an oven and shipped to the Institute of Marine Science, University of Alaska, Fairbanks, Alaska, for analyses of $\delta^{13}C$, $\delta^{15}N$, %C, and %N with a Europa GC-MS NACA system. The isotope value is expressed in the conventional delta (δ) notation, defined as the per mil (‰) deviation from the isotope standard, Peedee Belemnite limestone (PDB) for $^{13}C/^{12}C$ and atmospheric N_2 for $^{15}N/^{14}N$. The analytical precision for $\delta^{13}C$ and $\delta^{15}N$ was within 0.2‰, based on repeat measurements of a secondary standard (N. Haubenstock, University of Alaska, personal communication).

Results and discussion

$\delta^{13}C$ measurements

The $\delta^{13}C$ of phytoplankton, zooplankton, gut contents, and muscle tissue of silver carp and bighead showed small variations within each fishpond, with ranges from 0.5 to 2.2‰ (Fig. 1A). Microscopic examinations of suspended organic matter and gut contents indicated the predominance of plankton biomass in the water column and fish diets. The $\delta^{13}C$ similarity among plankton, gut contents, and muscle tissue within

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Fig. 1. Carbon and nitrogen isotopes (mean + SD) of phytoplankton (PP), zooplankton (ZP), gut contents (GC), and muscle tissue of silver carp (SC) and bighead (BH) from five fishponds.

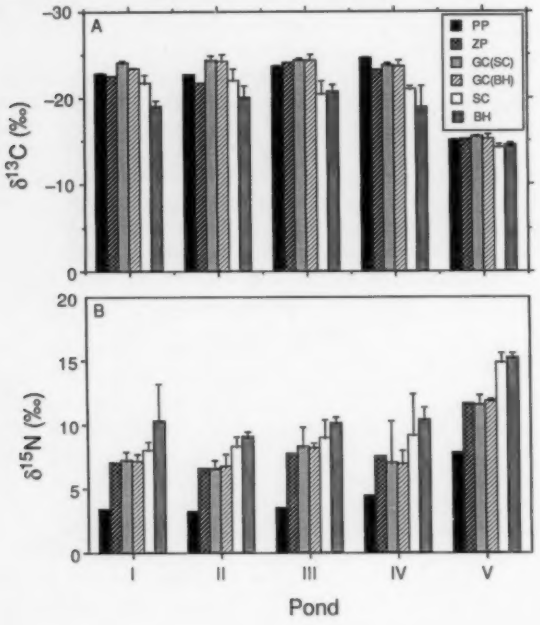
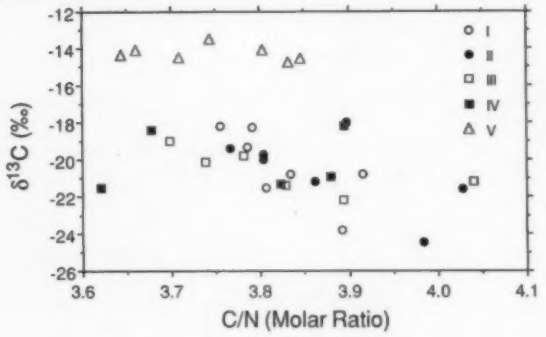


Fig. 2. Relationship between $\delta^{13}C$ and C/N ratio of muscle tissue of silver carp and bighead.



each fishpond suggests that both fish fed on plankton. Similarly, other carbon isotope analyses also indicate a dependence of silver carp and (or) bighead on plankton production in aquaculture ponds (Schroeder 1983*a*; Guo et al. 1987). It is unlikely that the fish derived their food from benthic algae and invertebrates because benthic organisms are often rare in polyculture ponds owing to the presence of several benthic omnivorous fishes, which is also the case for the study ponds. The C/N ratios of gut contents (7.4 ± 0.7 (mean ± SD), $n = 34$) and suspended organic matter (8.1 ± 1.9, $n = 17$) from the fishponds were significantly lower than those (>15) of terrestrial materials, indicating that terrestrial inputs to the fishponds were insignificant.

Differences in $\delta^{13}C$ between zooplankton and phytoplankton, and between gut contents of the two fish, were small and inconsistent (range from –0.4 to 1.0‰) within each fishpond (Fig. 1A). Therefore, it is not possible to use these values to estimate the relative importance of phytoplankton and zooplankton in supporting fish growth. Although muscle $\delta^{13}C$ of bighead was enriched by 0.4–1.5‰ relative to silver carp (Fig. 1A), these $\delta^{13}C$ values decreased with increasing C/N, except for the fish in pond V (Fig. 2), which, however, displayed similar muscle $\delta^{13}C$ values (–15.4 versus –15.2‰, mean for each species, see also Fig. 1A). Lipid is depleted in ^{13}C (Parker 1964), and an increase in C/N is generally attributed to increasing lipid content in muscle tissue (McConnaughey and McRoy 1979). The negative relationship between $\delta^{13}C$ and C/N indicates that lipid accumulation in muscle tissue significantly influenced muscle $\delta^{13}C$. Therefore, we refrained from using muscle $\delta^{13}C$ as an indicator of trophic level difference between the two species. Similar observations were documented from the Weddell Sea food webs (Rau et al. 1991, 1992).

$\delta^{15}N$ measurements

Large differences in $\delta^{15}N$ among phytoplankton, zooplankton, and fish muscle were found within each fishpond, with ranges from 5.8 (pond II) to 7.4‰ (pond V) (Fig. 1B). $\delta^{15}N$ for zooplankton was 3.1–4.2‰ higher than for phytoplankton. This is in good agreement with several studies on $\delta^{15}N$ enrichment factors specific to zooplankton (Montoya et al. 1990; Kling et al. 1992; Gu et al. 1994).

Muscle $\delta^{15}N$ values of silver carp and bighead were 4.6–7.0 and 5.8–7.4‰ higher than those for phytoplankton, respectively, and 1.0–2.2 and 2.5–3.6‰ higher than those for zooplankton, respectively (Fig. 1B). Unlike muscle $\delta^{13}C$, muscle $\delta^{15}N$ showed no significant correlation with C/N. Several studies indicate that $\delta^{15}N$ enrichments between fish and their diets typically range from 3.0 to 4.0‰ (Minagawa and Wada 1984; Wada et al. 1987; Fry 1988; Hobson and Welch 1992). The isotopic enrichments in silver carp and bighead suggest that the two fish fed on both phytoplankton and zooplankton. However, the nearly 3 or 4‰ enrichments in bighead relative to zooplankton imply that bighead fed largely on zooplankton. The gut content $\delta^{15}N$ values of silver carp and bighead were virtually identical and very similar to those for zooplankton within each fishpond (Fig. 1B). Because gut contents reflect recently acquired food and muscle tissue represents integrated feeding of animals (Hesslein et al. 1993), the identical $\delta^{15}N$ of gut contents between the two fish does not necessarily indicate long-term feeding similarity. This view is supported by the disparity of their muscle $\delta^{15}N$ values.

Dietary overlap

Trophic positions of aquatic animals can be determined by comparing $\delta^{15}N$ values of animals and their diets (Fry et al. 1988; Hesslein et al. 1991; Cabana and Rasmussen 1994). However, plankton $\delta^{15}N$ often varies seasonally (Gu et al. 1994), and our data were derived from a single sampling. We therefore focused our analysis on muscle $\delta^{15}N$, which reflects dietary isotope variation over time and can be a reliable trophic indicator. Because phytoplankton and zooplankton were the major food sources in our study ponds, a comparison of muscle $\delta^{15}N$ between the two fish may yield information on the degrees of dietary overlap. For example, muscle $\delta^{15}N$ will be

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Table 2. Mean dietary overlap between silver carp (SC) and bighead (BH) within each fishpond as estimated using differences in mean muscle $\delta^{15}N$ and three enrichment factors (a).

Pond No.	Muscle $\delta^{15}N$			Dietary overlap (%)		
	BH (x)	SC (y)	Difference ($x-y$)	$a=3.0$	$a=3.5$	$a=4.0$
I	10.3±2.9	8.0±0.7	2.3	23	34	42
II	9.0±0.4	8.3±0.7	0.7	76	80	82
III	10.1±0.5	8.9±1.5	1.2	60	65	70
IV	10.4±0.9	9.1±1.4	1.4	56	62	67
V	15.2±0.4	14.8±0.8	0.4	87	88	90

Note: Values for $\delta^{15}N$ are given as the mean ± SD.

identical if there is a complete dietary overlap; differences in muscle $\delta^{15}N$ will be intermediate if there is a partial overlap and large (3–4‰) if there is no overlap. An intrapond comparison of the mean muscle $\delta^{15}N$ reveals that there were consistent isotopic enrichments in bighead compared with silver carp (Table 2), likely indicating that the former fed at a higher trophic level than the latter. However, the enrichments were smaller than the 3–4‰ expected from feeding on two adjacent trophic levels, suggesting that there were dietary overlaps between the two species. We developed a mixing model to estimate the degree (f) of dietary overlap or similarity between silver carp and bighead:

$$f(\%) = (1 - (x - y)/a) \times 100$$

where x and y are the muscle $\delta^{15}N$ values for bighead and silver carp, respectively, and a is a $\delta^{15}N$ enrichment factor. We used literature values of 3.0, 3.5, and 4.0 reported for other fish species. Enrichment factors for silver carp and bighead are not available but are expected to fall into this range. Using this model, we estimated that the dietary overlap between silver carp and bighead ranged from 23 (pond I) to 87% (pond V), on the basis of an enrichment factor of 3.0. In four of the five fishponds, dietary overlap exceeded 50%, with an average of 60%. Using an enrichment factor of 3.0 or 4.0 resulted in estimate errors ≤10% (Table 2). These results strongly indicate that there were some severe dietary overlaps between the two planktivores in our study ponds and contradict a previous finding (Nie and Chiang 1954; Cremer and Smitherman 1980) that silver carp and bighead feed at different trophic levels. Because bighead fed on zooplankton, the dietary overlap suggests that silver carp depended heavily on zooplankton as well.

The variations in dietary overlap among the fishponds cannot be attributed to interspecific differences in isotope fractionation during nitrogen assimilation. Changes in muscle $\delta^{15}N$ during fish growth have been reported (Estep and Vigg 1985; Hesslein et al. 1993). A comparison of muscle $\delta^{15}N$ between the two species at different growth stages can be misleading. However, the total length and carbon and nitrogen contents of the two fish were similar within each fishpond (Table 1). There was no relationship between total length of each fish and their respective muscle $\delta^{15}N$. Variations in stocking density among fishponds may also influence the muscle $\delta^{15}N$ via food competition. However, stocking densities of the two fish were similar among the fishponds (X. Huang, Zhanjiang Fisheries College, personal communication). It is likely that the varying differences in $\delta^{15}N$ between the two fish (and the degrees of dietary overlap) reflected the variation in zooplankton availability among fishponds. Bighead are more efficient in obtaining zooplankton than silver carp (Nie and Chiang 1954). Pond to pond variation in the relative abundance of zooplankton in the diet of silver carp can be expected if the zooplankton availability was limiting. Additional data for plankton biomass are needed to verify this hypothesis.

The heavy dependence of silver carp on zooplankton evidenced from our results is supported by several recent studies indicating that zooplankton could be an important diet for silver carp. Zhuo and Lin (1990) showed that the ratio (wet biomass) of phytoplankton to zooplankton in the guts of silver carp was 1:47.6, indicating a predominance of zooplankton in the diets. Fish farmers in the Pearl River Delta, China, have long observed that the growth of bighead is often inhibited by silver carp in polyculture ponds (Huang and Gu 1979). Bitterlich and Gnaiger (1984) demonstrated experimentally that zooplankton were lysed within 20 min of incubation with gut fluid of silver carp and became indistinguishable from detritus. Therefore, the absence of zooplankton in the foregut of silver carp does not indicate a selective phytoplanktivorous strategy. It is not difficult to understand the preference of zooplankton over phytoplankton by silver carp because zooplankton contain significantly more calories than phytoplankton (Wang 1988) and because silver carp have difficulty obtaining nutrients from some phytoplankton as a result of the lack of cellulase enzymes in their digestive system (Gerking 1994). A recent carbon isotope study revealed that anchovy (*Engraulis capensis*) from southern Benguela were largely zooplanktivorous, rather than phytoplanktivorous as originally concluded (Monteiro et al. 1991).

Our results suggest that there was a considerable dietary overlap between silver carp and bighead in our study ponds. Conventional dietary analyses might have underestimated the nutritional importance of zooplankton in silver carp because of their inability to determine the dietary components incorporated into fish tissue and to determine dietary changes over time. The effect of silver carp on the growth of bighead previously observed in polyculture ponds is probably direct, i.e., competition for food organisms of the same trophic levels. Our results may also shed light on why silver carp fail to control algal blooms but enhance primary production in lakes. Trophic relationships between silver carp and bighead may be different in other aquaculture systems where food sources other than plankton (i.e., plant detritus and artificial feed) are also available for fish consumption. Furthermore, the relative importance of zooplankton in the diet of silver carp may change with growth (Miura 1990). Future research is needed for a better understanding of the nutritional role of phytoplankton and zooplankton in silver carp and bighead at different growth periods.

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Multiple effects of planktivorous fish on algae through a pelagic trophic cascade

Charles W. Ramcharan, Robert L. France, and D.J. McQueen

Abstract: We used a 2 × 2 factorial design enclosure study of biomanipulation (controls, with fish, with added nutrients, and with both fish and nutrients) during 1987 in Lake St. George, Ontario, to determine the impacts of planktivorous fish on phytoplankton populations and water clarity. Addition of nutrients had little or no effect on either communities of zoo- or phyto-plankton, or water clarity, but addition of fish caused dramatic changes. Fish biomass was negatively related to daphnid biomass and body size, but was positively related to bosminid and rotifer biomass. Fish also caused a marked shift in algal communities towards smaller cells and reduced water clarity. However, neither fish nor nutrient treatments had significant effects on concentrations of chlorophyll *a*. Our estimates of fish consumption and excretion rates and zooplankton community grazing and excretion rates indicated that fish-induced reductions in zooplankton biomasses were indirectly responsible for changes in the phytoplankton. By reducing herbivory, fish caused decreases in both zooplankton grazing and nutrient recycling. Our results show that changes in fish communities can influence water clarity, but that the simple trophic cascade is confounded by several other direct and indirect mechanisms involving shifts in grazing pressure, nutrient excretion, and phytoplankton cell size.

Résumé : Nous avons réalisé une étude de biomanipulation en enclos (témoins, avec poissons, avec ajout de nutriants, et avec à la fois poissons et nutriants) selon un plan factoriel de 2 × 2 pour mesurer, en 1987, dans le lac St. George (Ontario), les effets des poissons planctonophages sur les populations de phytoplancton et sur la transparence de l'eau. L'ajout de nutriants n'a eu pratiquement aucun effet sur les communautés de zoo- ou de phyto-plancton, ni sur la transparence de l'eau, mais l'introduction de poissons a causé des changements spectaculaires. La biomasse de poissons était en relation négative avec la biomasse et la taille corporelle des daphnidés, mais positivement corrélée à la biomasse de bosminidés et de rotifères. La présence des poissons a aussi causé une modification marquée des communautés algales, qui s'est traduite par des cellules plus petites et par une diminution de la transparence de l'eau. Toutefois, ni l'introduction de poissons ni l'ajout de nutriants n'a eu d'effets significatifs sur les concentrations de chlorophylle *a*. Nos estimations des taux de consommation et d'excrétion des poissons, et des taux de broutage et d'excrétion de la communauté zooplanctonique, indiquent que les réductions de la biomasse zooplanctonique induites par les poissons étaient indirectement responsables des changements survenus dans le phytoplancton. En réduisant l'activité des herbivores, les poissons ont causé des baisses tant du broutage par le zooplancton que du recyclage des nutriants. Nos résultats montrent que les changements dans les communautés de poissons peuvent influer sur la transparence de l'eau, mais que la simple cascade trophique est compliquée par divers autres mécanismes directs et indirects impliquant des changements dans la pression de broutage, l'excrétion de nutriants et la taille des cellules du phytoplancton.
[Traduit par la Rédaction]

Introduction

The effects of planktivorous fish on phytoplankton are complex. Fish can influence algae directly through nutrient regeneration and indirectly through impacts on zooplankton populations (Carpenter et al. 1993). For example, the biomanipulation hypothesis postulates that increased fish planktivory should enhance algal populations by decreasing zooplankton grazing (Shapiro and Wright 1984; Carpenter and Kitchell 1988). Planktivorous fish would, therefore, have a positive effect on algal biomass. Another type of positive impact of fish on algae results from nutrient excretion. By excreting nitrogen and phosphorus (P) (Lamarra 1975; Nakashima and Leggett 1980; Tátrai and Istvánovics 1986; Braband et al. 1990) through their feeding, metabolism, and death (Threlkeld 1987), fish can promote the growth of algal populations (e.g., Drenner et al. 1986, 1989; Threlkeld 1988; Vanni and Findlay 1990; Schindler 1992; Carpenter et al. 1993). A third possibility is that planktivorous fish may have no effect, positive or negative, on algal populations because primary productivity may be controlled by nutrient loading (bottom up) (McQueen and Post 1988; McQueen et al. 1989).

In addition to the two known effects of planktivorous fish on algae (i.e., mediated through zooplankton grazing or fish nutrient recycling), we suggest that fish can also affect primary productivity through changes in zooplankton nutrient recycling. Like fish, zooplankton are important sources of nutrient resupply to algae (e.g., Peters 1987; McCauley and Kalff 1987; Scavia 1979; Taylor and Lean 1991). By reducing zooplankton biomass, planktivorous fish may indirectly reduce the total

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Table 1. Schedule of fish additions and estimates of fish population size in each of the four enclosures with fish.

Enclosure	Date	Fish numbers	Mean mass (g)	Total biomass (g)
F (1)	May 13	65	2.95	191
	July 13	61	5.50	336
	Aug. 27	23	11.30	260
F (2)	May 13	65	3.06	198
	July 13	51	5.86	299
	Aug. 27	38	10.24	389
NF (1)	May 13	65	2.91	189
	July 13	43	4.96	214
	Aug. 27	6	10.10	61
NF (2)	May 13	68	2.99	203
	July 13	65	4.70	306
	Aug. 27	25	10.80	207

Note: Treatments with only fish added are indicated by F, and those with both nutrients and fish are indicated by NF. Numbers in parentheses are replicate numbers.

recycling of nutrients to algae. In contrast to the trophic cascade hypothesis and the effects of fish on nutrient recycling, by reducing zooplankton nutrient recycling the impact of planktivorous fish on algae would be negative.

In this study we tested the impacts of planktivorous fish on algae as mediated by three different mechanisms: (*i*) reduction in zooplankton grazing via the trophic cascade, (*ii*) nutrient recycling from fish, and (*iii*) reduction in nutrient recycling from zooplankton. We used large enclosures to analyze in detail the effects of planktivorous fish on limnetic food webs. Because the importance of fish and zooplankton as nutrient sources for algae may depend on overall system productivity, our experiments were conducted under mesotrophic (without added nutrients) and eutrophic (with added nutrients) conditions. For 20 weeks we monitored biomasses of fish, zooplankton, and algae. We used mathematical estimates of the rates and pathways of energy and nutrient flow, to identify the direct and indirect effects of fish and zooplankton.

Our analyses highlight the complexity of the final link in the trophic cascade, between zooplankton and phytoplankton (McQueen and Post 1988; McQueen et al. 1989; Carpenter et al. 1993); our approach of modeling food web dynamics allowed us to account for the fact that (*i*) fish affect algal populations through several simultaneous mechanisms that are mediated through nutrient dynamics as well as zooplankton grazing (Carpenter et al. 1993), (*ii*) algal communities respond to recycled nutrients and to zooplankton herbivory by changing size distribution (e.g., McQueen et al. 1992), species composition, and biomass (Carpenter et al. 1987), (*iii*) although planktivorous fish may strongly affect populations of zooplankton grazers, the transmission of those effects through to algae is confounded by changes in nutrient flux caused by all fish and especially by zooplankton (McQueen et al. 1992; McQueen and Post 1988; Carpenter et al. 1993).

Methods

Enclosures

Enclosure experiments were conducted at Lake St. George, a mesotrophic kettle lake approximately 40 km north of Toronto, Ontario (McQueen et al. 1989). Experimental design, descriptions of the enclosures, and field sampling methods are similar to those of McQueen et al. (1992). We used eight enclosures that were 8 m in diameter and 14 m deep and each contained approximately 700 m^3 of lake water. During April 1987, the side walls were raised for 2–3 days to allow lake water to circulate. The walls were then lowered and secured by SCUBA divers, which took several days. To ensure that all of the enclosures began with the same initial water conditions, we added approximately 600 m^3 of epilimnetic lake water to each enclosure using a high-capacity pump. To initially remove all fish from the enclosures, we used a 30 m long × 10 m deep purse seine (1.5 cm stretch mesh) to fish the enclosures until no more fish were caught. Throughout the experiment we used fish traps to ensure that the fish-free treatments remained fishless.

Experimental design

A factorial design with two replicates was used. The treatments were as follows: unfertilized, fishless controls (C), nutrient enrichment without fish (N), fish without nutrient enrichment (F), and nutrients plus fish (NF) (eight enclosures total). Previous reports based on these experiments (e.g., Mazumder et al. 1990*a*, 1990*b*) concerned the analysis of particle size data that were independently collected and did not involve data from the weekly sampling program that are presented here.

Nutrient additions began on 6 May 1987 and continued each week for 15 weeks through 17 August 1987. On each occasion, nutrients were added as single applications (3.05 g H_3PO_4 and 76.22 g $NaNO_3$). Our applications were intended to maintain the N and NF enclosures as eutrophic treatments and the C and F enclosures as more oligotrophic or mesotrophic treatments. They were also intended to maintain the N:P ratio at approximately 13:1.

On 13 May, age 1+ yellow perch were added to four enclosures (Table 1). All of these fish were captured (purse nets and beach seine) from Lake St. George and were weighed and measured (total length) before being introduced to the enclosures. Fish were censused by mark–recapture using a purse seine on 13 July and at the end of the experiment on 27 August. The fish were weighed and measured then returned to the enclosure for recapture 3 days later.

Weekly sampling

Sampling for zooplankton, phytoplankton, chlorophyll *a*, water chemistry, Secchi depth, oxygen, temperature, and light began on 11 June 1987 and continued weekly to 17 August 1987. The zooplankton were sampled at 1 m depth intervals from 1 to 5 m, and at 2 m depth intervals from 7 to 13 m using a 35-L Plexiglas plankton trap (Schindler 1969) equipped with a 40-μm Nitex plankton net. For each enclosure, the weekly samples were combined to yield two samples representing the epilimnion (1–5 m) and the hypolimnion (6–13 m). These composite samples were counted (numbers and biomass) using a computer-assisted zooplankton counting and measuring device (Sprules et al. 1981). The zooplankton groups that were enumerated included (*i*) cyclopoid copepod adults and copepodids comprising mainly *Diacyclops bicuspidatus thomasi* and *Mesocyclops edax*, (*ii*) calanoid copepod adults and copepodids including mainly *Skistodiaptomus oregonensis* and *Skistodiaptomus minutus*, (*iii*) daphnids including mainly *Daphnia galeata mendotae*, *Daphnia retrocurva*, and *Ceriodaphnia* sp., (*iv*) rotifers comprising mainly *Keratella cochlearis*, *Filinia longiseta*, *Polyarthra* sp., and *Asplanchna* sp., and (*v*) bosminids comprising mainly *Bosmina longirostris* and *Eubosmina coregoni*. Water chemistry samples were collected with an integrated tube sampler (0–4 m) and the samples were analyzed at the National Water Research Institute, Burlington, Ontario (Aspila et al. 1976).

Algal samples were collected weekly using an integrated tube sampler deployed between 0 and 4 m depth. The samples were preserved in Lugol's iodine and stored in glass bottles in the dark. For each enclosure, Utermohl (1958) counts were made from samples collected on 25 May, 6 July, 4 August, and 17 August 1987. Cells

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were identified to genus, or species where possible, and cell lengths and widths were recorded. In later analyses, cell types were grouped as edible and inedible on the basis of the criteria described by McQueen and Post (1988).

Fish consumption and nutrient excretion

We estimated the rate of P excretion by fish with a bioenergetics-based technique (Kraft 1992; McQueen et al. 1992), where

$$P_{\text{eliminated}} = P_{\text{consumed}} - P_{\text{growth}}$$

The amounts of food that were consumed, used in growth, and eliminated were calculated using Hewett and Johnson's (1992) bioenergetics computer program. We used the bioenergetics model to calculate the mass of prey that the fish would have needed to consume while growing at our observed rates. Values for physiological constants needed by the model were taken from Hewett and Johnson's (1992) data for yellow perch. The assumptions for the bioenergetics model were that the fish remained in the epilimnion, experienced epilimnetic temperatures, and ate only zooplankton prey. The bioenergetics model provided estimates of fish consumption, respiration, egestion, and excretion in units of grams of prey wet mass per gram of predator wet mass each day. Before calculating P excretion with eq. 1, the outputs of the bioenergetics model were converted to P using monthly measurements of the P content of fish and their zooplankton prey (particles >400 μm) taken from Mazumder (1988) and Mazumder et al. (1988). To express fish P excretion in the same units as zooplankton excretion (mg $P{\cdot}L^{-1}{\cdot}day^{-1}$), we multiplied the number of fish in each enclosure by the average perch mass and divided by the volume of the epilimnion (1.6×10^4 L, with an average of 4.0 m mixed depth).

Fish that died during the experiment either sank out of the epilimnion or floated and were removed without being replaced. In either case, dead fish were not an additional source of P. We estimated the number of live fish in the enclosure on each day of the model run by interpolating between the start, middle (from the mark–recapture on 13 July), and end values.

Zooplankton grazing and nutrient excretion

Zooplankton grazing rates were estimated using published linear regression models. Only equations developed from in situ studies of zooplankton herbivory (Bogdan et al. 1980; Chow-Fraser and Knoechel 1985; Chow-Fraser 1986) were used, because models developed from laboratory studies tend to underestimate zooplankton grazing (Knoechel and Holtby 1986). Except for rotifers, all equations to estimate grazing rate were of the following form:

$$\log_{10}(\text{CR}) = a \log_{10}(\text{BL}) + b$$

where CR is the clearance rate (millilitres per day), BL is body length (millimetres), and a and b are parameters of the least-squares regression.

Values of a and b for different zooplankton species were taken from Chow-Fraser (1986) and Chow-Fraser and Knoechel (1985). To correspond with our zooplankton data, values for a and b were averaged within the following taxa: Daphnidae, Bosminidae, and Diaptomidae. Rotifer clearance rates were estimated using the averaged values of Bodgan et al. (1980) for rotifers feeding on *Chlamydomonas* spp.

For epilimnetic zooplankters, clearance rates (millilitres per animal each day) were multiplied by the density (animals per litre) of each taxon and summed to obtain epilimnetic clearance rates (millilitres per litre each day). For hypolimnetic grazers, we assumed that substantial algal food was available only in the epilimnion and that zooplankters migrated to the upper waters at night to feed (Stirling 1994). We set their daily feeding period at 7 h. Calculated clearance rates (millilitres per animal each day) were multiplied by 7/24, multiplied by animal density, and then added to the rate for epilimnetic animals to obtain a total community clearance rate (millilitres per litre each day).

We modeled P release from herbivorous zooplankton as a function of grazing rate in a manner similar to Vanni and Findlay (1990). In their paper, Vanni and Findlay (1990) used estimates of grazing rates to calculate P ingestion rate, with necessary assumptions about algal biomass: carbon and carbon:phosphorus ratios. Our estimates of P excretion were more straightforward, because data on size-fractioned P from our mesocosms had already been published by Mazumder et al. (1988). Because Mazumder et al. (1988) sampled the enclosures for a shorter period (17 June to 18 August) than we did (11 May to 24 August), our estimates of zooplankton P excretion extend for a shorter sampling period than the rest of our study. For each sampling day, zooplankton grazing rate (millilitres per animal per day) was multiplied by the concentration of P (nanograms of P per millilitre) found in edible particles (<20 mm), which gave the rate of P ingestion (nanograms of P per litre per day). We then assumed that 50% of ingested P was excreted (Peters and Rigler 1973).

For carnivorous zooplankton and nauplii, nutrient excretion was estimated by multiplying Taylor and Lean's (1991) in situ measurements of P excretion for various zooplankton taxa by the density of those taxa in our enclosures. As a test of the accuracy of this simple technique, we calculated P excretion by zooplankton grazers using both methods, and obtained surprisingly similar results. To calculate community excretion rates for the whole water column the same technique used for grazing rates was applied: hypolimnetic excretion rates (nanograms of P per animal each day) were multiplied by 7/24, multiplied again by animal density, and added to the epilimnetic rates.

Statistical analyses

Model I, two-way analyses of variance with repeated measures were applied to the main effects ((*i*) nutrient, (*ii*) fish, and (*iii*) nutrient × fish interactions) and the time trends ((*i*) time × nutrients, (*ii*) time × fish, and (*iii*) time × nutrients × fish). The groups tested were the biomasses of total epilimnetic zooplankton, daphnids, bosminids, calanoids, cyclopoids, nauplii, rotifers, and *Asplanchna*; the individual lengths of total epilimnetic zooplankton, daphnids, bosminids, calanoids, and cyclopoids; and Secchi depth and chlorophyll *a* concentrations. Bonferroni's correction for multiple comparisons was used to reduce the probability of type II errors.

Analyses of main effects and their interactions were based on comparisons of treatment responses over all sampling intervals. Orthogonal a priori planned comparisons were undertaken whenever ANOVA revealed significant main (between subject) effects to determine which treatments were significantly different from each other: (*i*) if nutrient between-subject effects were significantly different then C versus N and F versus NF contrasts were examined, (*ii*) if fish between-subject effects were significantly different then C versus F and N versus NF contrasts were examined, and (*iii*) if nutrient–fish between-subject interactions were significantly different then C versus NF and N versus F contrasts were examined. In the present analysis, if treatments differed in the pattern of their responses over time (i.e., within-subject effects; Winer 1971), the appropriate contrasts were examined. If no linear differences were observed between the contrasts, quadratic and cubic contrasts were examined (Gurevitch and Chester 1986). All zooplankton data represent values calculated for the whole water column and were log-transformed to reduce heteroscedasticity before ANOVAR.

Results

Standing stock data

Mean fish biomasses (Table 1) in the two fish treatments were 65.6 (enclosure F1) and 73.9 $kg{\cdot}ha^{-1}$ (enclosure F2), and in the two nutrient plus fish treatments they were 39.5 (enclosure NF1) and 65.0 (enclosure NF2) $kg{\cdot}ha^{-1}$. All of these biomasses

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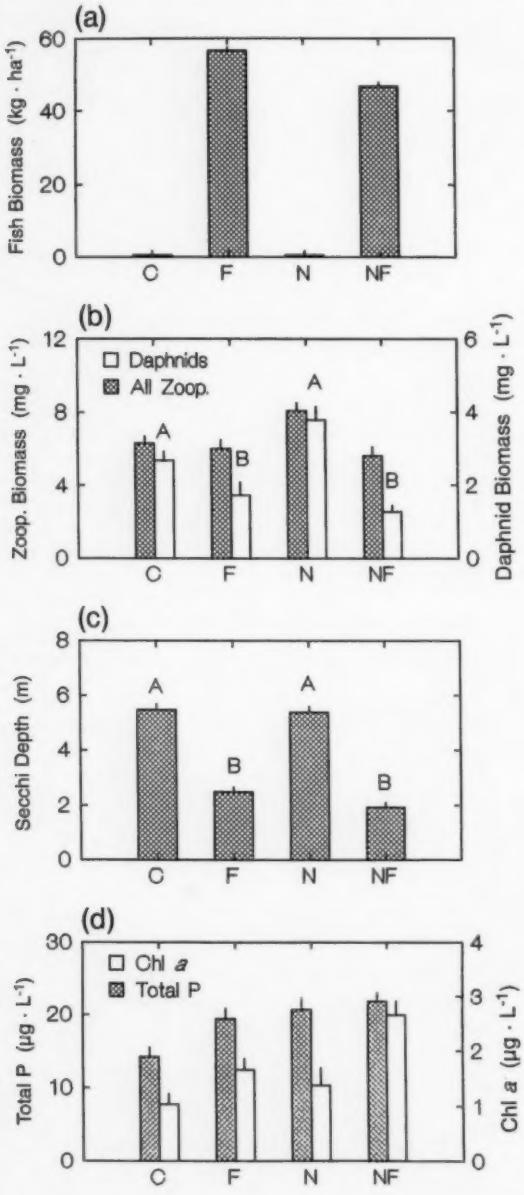
Fig. 1. Mean annual responses of the biomasses of fish, zooplankton (whole water column), daphnids, and phytoplankton, the depth of Secchi transparency, and the concentrations of chlorophyll *a* and total P to the experimental treatments: control (C), nutrients (N), fish (F), and nutrients plus fish (NF). In *b*, total zooplankton biomass is represented by the hatched bars and daphnid biomass by the open bars. In *d*, total P concentrations are represented by the hatched bars and chlorophyll *a* concentrations by the open bars. The letters over the bars in *b* and *c* indicate groups that are statistically similar (Newman–Keuls test, $p < 0.05$, Zar 1984).



were within natural carrying capacity (between 20 and 200 kg·ha^{-1}) on the basis of 9 years of continuous food web monitoring at Lake St George. The fish biomasses (Fig. 1) in the nonfish treatments (C and N) never exceeded 1 kg·ha^{-1} and comprised invaders that occasionally jumped into the enclosures; these were trapped and removed on a daily basis.

Overall trends

The presence of fish had dramatic impacts on populations of zooplankton throughout the water column. Total zooplankton and especially daphnid biomasses in the nonfish (C and N) treatments were higher than in the treatments that contained fish (F and NF) (Fig. 1). The biomasses of smaller grazers, such as bosminids, calanoids, nauplii, and rotifers, tended to be enhanced by the presence of fish. The effects of fish were strongest in the epilimnion, where daphnid biomasses crashed (Fig. 2), and body sizes of daphnids, calanoids, and bosminids were all reduced. Mean annual estimates of Secchi depth and chlorophyll *a* suggested that the nonfish treatments had clearer water and lower algal biomass (Fig. 1). Mean annual estimates of total P revealed a trend from the lowest values in the controls to the highest values in the nutrient plus fish (NF) treatments (Fig. 1).

The phytoplankton size spectrum changed dramatically with respect to treatment (Fig. 3). The main effect was a decrease in the abundance of large cells in the fish treatments and especially in the NF treatments. These treatments also had substantial increases in total concentrations of small particles (greatest axial dimension 1–5 and 5–20 µm). Moreover, the fish treatments had increases in the percentage of inedible chlorophytes, primarily mucilaginous sheathed algae such as *Gloeococcus* spp. (McQueen and Post 1988). Seasonal averages for the percentage of inedible chlorophytes in the 1- to 20-µm size-class for each treatment were as follows: C, 1.3%; N, 1.1%; F, 13.7%; and NF, 5.6%.

Statistical analyses

In terms of the main effects, nutrient treatment had no effects on any of the groups (p values from 0.201 to 0.959), and there were no nutrient × fish interactions (ANOVA, p values from 0.131 to 0.933). In contrast, the fish additions decreased the biomasses of daphnids ($p < 0.01$) and also decreased the body lengths of both bosminids ($p < 0.009$) and daphnids ($p < 0.006$). The presence of fish decreased Secchi depth ($p < 0.0005$) but had no effect on chlorophyll *a* concentration ($p > 0.05$, Figs. 1, 4). In terms of time trends, several variables showed changes throughout the season: the biomass of bosminids, calanoids, cyclopoids, and daphnids; and the body lengths of these same taxa (p values from <0.01 to <0.0001). There were, again, no significant effects of nutrient additions (i.e., no time × nutrient effects). The fish additions were associated with continuous decreases in the lengths of calanoids through time (i.e., a time × fish effect) ($p < 0.009$). There was also a significant decrease in Secchi depth with time (Fig. 4) ($p < 0.0001$).

Using linear regression we found a negative trend between fish biomass and total zooplankton biomass ($r^2 = 0.33$, $p > 0.05$), and a positive trend between total zooplankton biomass and Secchi depth ($r^2 = 0.26$, $p > 0.05$, Fig. 5). The effects of fish biomass on daphnid biomass ($r^2 = 0.51$, $p < 0.03$) and of daphnid biomass on Secchi depth ($r^2 = 0.66$, $p < 0.01$) were

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Fig. 2. Daphnid biomass (log_{10} scale) on different sampling dates in the epilimnion (*a*), hypolimnion (*b*), and whole water column (*c*). Values are means for the two replicate enclosures. Circles are controls, triangles are nutrient treatments, squares are fish treatments, and diamonds are nutrient plus fish treatments. Open symbols show the treatments without fish and solid symbols the enclosures with fish.

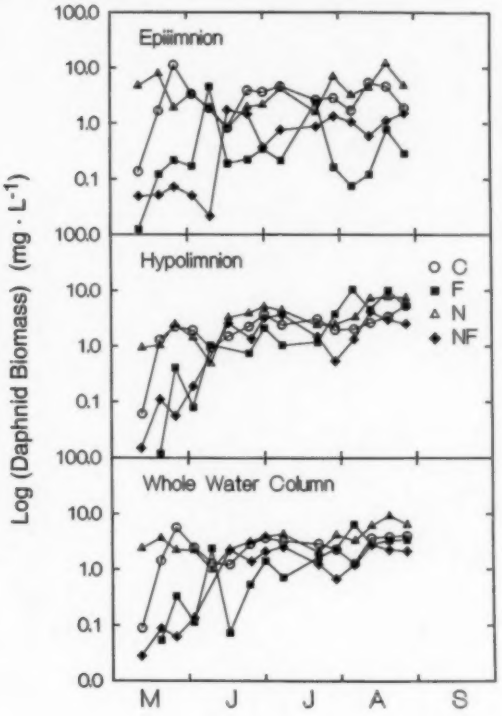
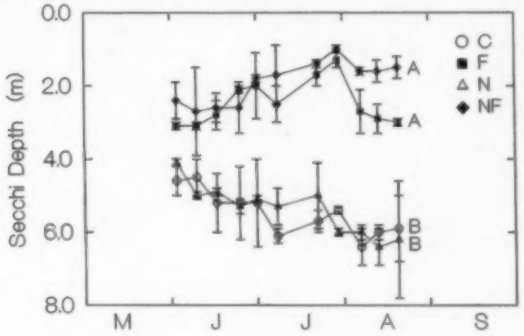


Fig. 3. Secchi depth in the control and treatment enclosures on different sampling dates. Values are means for the two replicates and error bars show the range. Circles are controls, triangles are nutrient treatments, squares are fish treatments, and diamonds are nutrient plus fish treatments. Open symbols show the treatments without fish and solid symbols the enclosures with fish. The letters A and B indicate groups that are statistically similar (Newman–Keuls test, $p < 0.05$, Zar 1984).



stronger than for the whole zooplankton community. There was also a negative relationship between fish biomass and Secchi depth per unit total P ($r^2 = 0.59$, $p < 0.02$). These results were congruent with results from similar enclosure experiments conducted during 1986 (McQueen et al. 1992). The relationship between fish biomass and the concentration of chlorophyll *a* per unit total P was not significant either in 1986 (McQueen et al. 1992) or in the current experiments ($r^2 = 0.02$, $p > 0.09$). Total epilimnetic P increased with chlorophyll *a* concentration, but not significantly ($r^2 = 0.33$, $p > 0.05$).

Rates of consumption and excretion

Zooplankton community grazing rates were highest in the treatments without fish (C and N) (Fig. 6). In all enclosures, community grazing rates varied through the year with fluctuations in the body size and abundance of daphnids, the most important herbivores. At peaks in grazing rate, herbivores in the C and N enclosures filtered a volume equivalent to the epilimnion more than once a day.

Zooplankton P excretion rates were primarily determined by daphnid biomass, which was higher in the treatments without fish (C and N) (Fig. 1). Excretion rates also changed through the season, with the highest rates occurring in the spring. The mean annual zooplankton excretion rates (Fig. 7) were lower in the two treatments that contained fish (F and NF).

Mean annual fish excretion rates (Fig. 7) were almost identical to those observed in 1986 (McQueen et al. 1992), and more than an order of magnitude lower than the rates observed for zooplankton. They were much less than the rate (0.3 mg $P \cdot L^{-1} \cdot day^{-1}$) that was loaded into the nutrient addition treatments (N and NF). The total recycling rates were higher in the enclosures that lacked fish (C and N).

In summary, the presence of planktivorous fish was associated with (*i*) reductions in zooplankton size, biomass, and numbers, especially in the epilimnion, (*ii*) a shift in algal size distribution towards smaller cells, (*iii*) decreased Secchi depths, (*iv*) no significant changes in the concentration of chlorophyll *a* per unit P, (*v*) reduced rates of zooplankton community grazing, (*vi*) reductions in P excretion rates from zooplankton, and (*vii*) relatively minor increases in the P excretion rates from fish.

Discussion

Results from both the current study and similar experiments that were conducted in 1986 (McQueen et al. 1992) illustrate that fish may have several different, simultaneous influences on phytoplankton populations and water clarity. The 1986 enclosure experiments in Lake St. George were a good example of the enhancement of algal populations by nutrients recycled from fish. In that study, planktivorous fish did not reduce either the biomass or the average body size of zooplankton (McQueen et al. 1992). A biomanipulation effect resulting from suppression of zooplankton grazing was therefore not a tenable hypothesis. Nevertheless, the presence of fish did reduce mean Secchi depth, but without affecting concentrations of chlorophyll *a*. Increased nutrient availability through recycling by fish was associated with a shift towards smaller algal cells (McQueen et al. 1992). The smaller cells caused increased turbidity (decreased Secchi depth), but there was no

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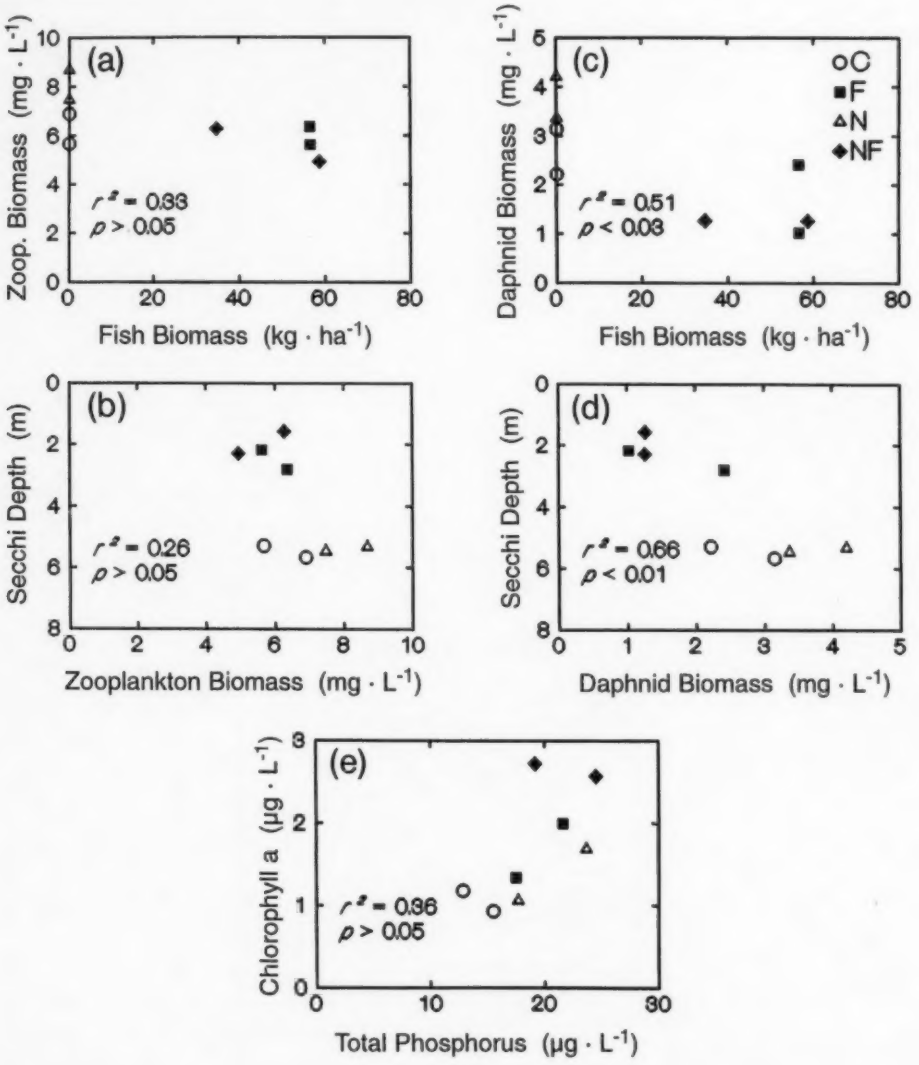
Fig. 4. Relationships between fish and zooplankton biomasses (*a* for all zooplankton, *c* for daphnids only), zooplankton biomass, and Secchi depth (*b* for all zooplankton, *d* for daphnids only), and concentration of total P and chlorophyll *a* (*e*). Values are annual means for each of the two replicates. C, controls; N, nutrients; F, fish; NF, nutrients plus fish. Open symbols show the treatments without fish and solid symbols the enclosures with fish.



overall change in chlorophyll *a* concentration, as would be expected from the trophic cascade hypothesis. The 1986 enclosure work supported the findings of Vanni and Findlay (1990), Drenner et al. (1989), Threlkeld (1987), Schindler (1992), and others in highlighting the importance of fish as sources of nutrient regeneration.

Our present results are in some ways similar to the 1986 study. The presence of planktivorous fish reduced mean Secchi depth but did not affect concentrations of chlorophyll *a* (see also France et al. 1992). Fish were also associated with a reduction in the number of larger algal cells. In contrast to the 1986 experiments, planktivorous fish reduced epilimnetic zooplankton biomass, abundance, and body size and also significantly decreased community grazing rates. Therefore, in addition to the mechanisms by which fish could have affected algae through their own nutrient regeneration, fish could also have indirectly affected algae through their impacts on zooplankton grazers.

In the fishless enclosures (C and N), large zooplankton reached high biomasses and abundances. Both our estimates of grazing rates and the independent measurements of Mazumder et al. (1990*a*) show that herbivory was increased by a factor of 4 compared with the enclosures with fish. High herbivory reduced populations of small, edible algal cells. The

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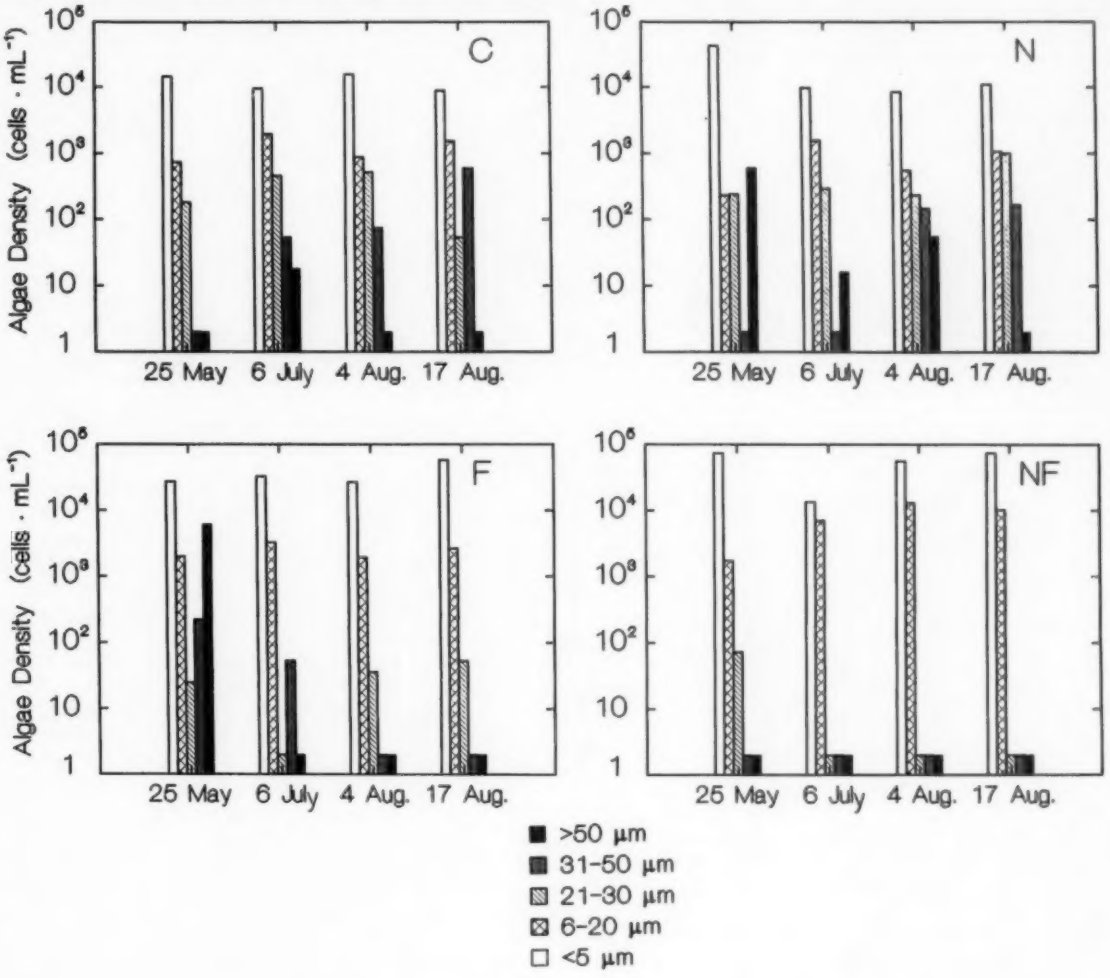
Fig. 5. Algal size distributions in the C, N, F, and NF treatments. Numbers of algal cells in five different size categories based on maximum cell length (μm) were averaged for the two replicates in each treatment.



enclosures with fish (F and NF) had dramatic reductions in zooplankton biomass, body size, and abundance. Herbivory on small, edible algae was relaxed.

Because the presence of fish was associated with reduced zooplankton grazing and higher densities of edible algae, a trophic cascade is a tenable hypothesis. However, the confounding effects of nutrient excretion by fish and zooplankton need to be considered. In some studies such as our 1986 enclosure experiments (McQueen et al. 1992), nutrient recycling had more substantial effects on algae than grazing. Our present estimates of rates of consumption and excretion by fish and of rates of excretion by zooplankton indicate that P was recycled by both zooplankton and fish, but that the primary nutrient source was zooplankton. P regeneration from zooplankton was also much higher than our experimental additions of nutrients, which may explain why the nutrient treatments had no detectable effect on the enclosures. The negative impacts of fish on zooplankton biomass caused an overall reduction in total P recycled by consumers in the F and NF enclosures. Mazumder et al. (1988, 1989) also found that nutrient uptake rates by algae were higher in the fishless enclosures. This fish-induced decrease in P recyling happened despite the shift in zooplankton size distributions towards smaller animals. Smaller zooplankters have higher mass-specific excretion rates (Bartell 1981), but this was not enough to offset the reduction in total zooplankton biomass caused by fish predation.

The difference in nutrient supply between the enclosures with and without fish would have contributed to the differences in phytoplankton size distribution. Large algal cells are poorer competitors for nutrients than small cells (e.g., Suttle et al. 1988). Therefore, large cells had a double advantage in the fishless enclosures: competition with small cells for nutrients was reduced by herbivory, and P supply from zooplankton was high. In the presence of fish, reduced herbivory and lower nutrient supply strongly favored smaller algal cells. Very high densities of small algal cells reduced water clarity. Therefore,

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Fig. 6. Daily community grazing rates ($mL \cdot L^{-1} \cdot day^{-1}$) in the epilimnion (*a*) and whole water column (*b*). The values for the two replicates in each treatment were averaged. C, controls; N, nutrients; F, fish; NF, nutrients plus fish. Open symbols show the treatments without fish and solid symbols the enclosures with fish. (*c*) Annual mean clearance rates.

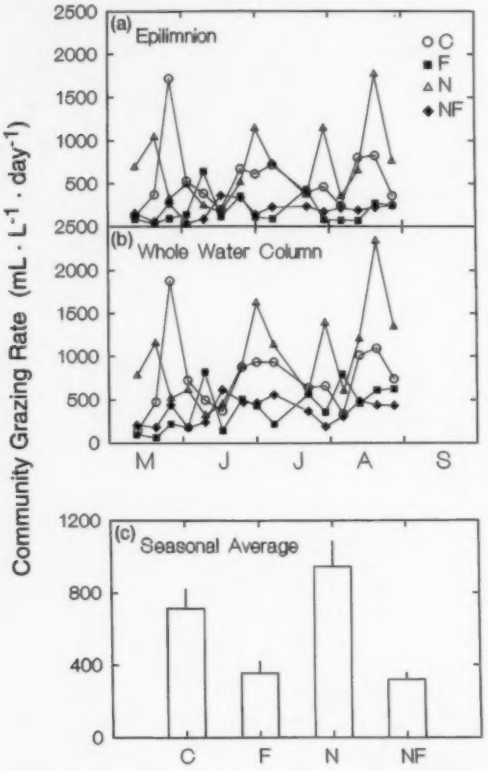
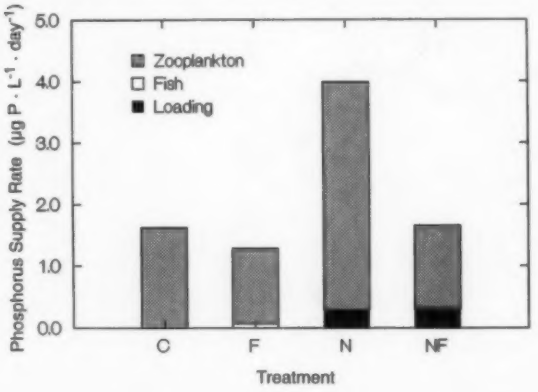


Fig. 7. Annual averages of daily phosphorus supply rates in the enclosures. There were three sources of P: zooplankton excretion (hatched bars), fish excretion (open bars), and external (experimental) nutrient loading (solid bars).



fish indirectly caused a reduction in Secchi transparency, yet without an overall change in chlorophyll *a* concentration (McQueen et al. 1992).

In whole food web experiments such as these that run for an entire summer season there are many complex direct and indirect interactions. Only an emphasis on ecological dynamics (on measuring or estimating rates of energy and nutrient flow) can reveal the relative importance of different mechanisms. The biomanipulation literature is heavily burdened with studies that report uncertainty about the zooplankton–phytoplankton link (De Melo et al. 1992). Inverse relationships between the biomasses of fish and daphnids are commonly found. However, strong disagreements exist about whether changes in daphnids either are transmitted (e.g., Vanni 1984) or become uncoupled (e.g., Noonan 1984; Drenner et al. 1986, 1990; Post and McQueen 1987; Threlkeld 1987; Threlkeld and Drenner 1987; McQueen and Post 1988; McQueen et al. 1989; Qin and Threlkeld 1990; Richardson et al. 1990) at the phytoplankton level. There are also disagreements about whether the few apparent top-down cascades that have been documented were caused by increased grazing or by changes in nutrient dynamics. In this study we have clearly shown that when planktivorous fish were absent, grazing from herbivorous zooplankton increased sufficiently to reduce populations of edible algae. The increased supply of nutrients from grazers could not offset the high grazing pressure, but would have benefitted larger, inedible algae. In the presence of fish, the combination of reduced grazing and increased total nutrient supply favored smaller algal cells.

In view of the small number of long-term studies that have unequivocally demonstrated fish-induced grazer effects on algal populations, these are surprising results! Most studies of the effects of planktivorous fish have been conducted in unstratified water bodies, either small enclosures or shallow lakes, and have run for only 2–4 months (DeMelo et al. 1992). Lack of a hypolimnion means that zooplankton have no refuge from fish predation, and pelagic nutrient processes such as sedimentation of particulate P cannot occur. Moreover, studies with small enclosures have substantial "wall effects" and sediment resuspension, and necessarily have very high fish biomasses. Several fish must be added to each treatment to reduce the effects of individual variability in fish feeding rates and to allow for mortality. In contrast, we have found evidence of fish-induced effects on algal populations in a more natural pelagic food web. Each of our thermally stratified enclosures contained natural biomasses of fish, zooplankton, and phytoplankton in 750 000 L of lake water.

The natural fish biomasses that were tractable in these large enclosures probably accounted for the reduction in biotic nutrient recycling caused by planktivorous fish. This reduction seems counterintuitive in light of the large number of studies that emphasize the importance of nutrients regenerated by fish themselves (e.g., Drenner et al. 1989; Braband et al. 1990; Vanni and Findlay 1990; Schindler 1992). Clearly the outcome of any experiment with fish depends on the biomass of fish used. In contrast to many other studies, we were careful to use fish biomasses that were well within the range normally found in our study lake (McQueen et al. 1992). Our experimental food webs were therefore not top heavy with fish. Another reason that the importance of zooplankton nutrient recycling relative to fish has not been recognized is that nutrient resupply by herbivores has not usually been measured or estimated in

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food web experiments (e.g., Reinertsen et al. 1986; Braband et al. 1990).

Some of the major difficulties in estimating biotic nutrient recycling are the assumptions that must be made about the P content of consumers and their prey. In contrast to most published estimates of nutrient excretion, our analyses were based on direct measurements of P concentrations in the fish, in the zooplankton prey eaten by the fish, and in the algae consumed by herbivorous zooplankton (Mazumder 1988; Mazumder et al. 1988). The only parameters in our P excretion models that were not directly measured were the P assimilation efficiencies of fish and zooplankton. The value used for fish (28%) was based on Kraft's (1992) model. Even if fish assimilation efficiency varied substantially from 28%, fish excretion was too low to be an important source of nutrients in our experiments compared with zooplankton (Fig. 7).

The P assimilation efficiency that we used for zooplankton (50%) was based on Peters and Rigler's (1973) model. Measurements of P assimilation efficiency conducted in situ have found values that vary around 50% (Taylor 1984; Taylor and Lean 1991), especially in P-limited systems such as our enclosures (Mazumder et al. 1988). Olsen et al. (1986) also found that P assimilation efficiency varied with the P:C ratio of ingested particles (Sterner 1986). They used enclosure experiments run for 8 days, during which time algae (low P:C ratio) gradually decreased, leaving mostly bacteria (high P:C ratio) as zooplankton food. In our enclosures, the diet of zooplankton herbivores may also have included relatively more bacteria as populations of edible algae were grazed-down in the C and N treatments. Since the P:C ratio of bacteria is usually higher than that of algae, we may have underestimated nutrient excretion in the C and N treatments. Therefore, the depressive effects of fish zooplankton nutrient excretion may have been stronger than estimated.

In summary, the enclosure experiments conducted in both 1986 (McQueen et al. 1992) and 1987 (the present study) suggest that the presence of planktivorous fish reduced Secchi transparency without affecting concentrations of chlorophyll *a*. These results were consistent between the 2 years, despite the fact that only in the current study did planktivorous fish reduce the biomass, body size, and abundance of zooplankton grazers. Overall, our estimates of rates of nutrient excretion by fish and zooplankton, and of zooplankton grazing, indicate that fish planktivory may (*i*) indirectly reduce herbivory on algal populations by depressing zooplankton grazers, (*ii*) substantially reduce nutrient resupply to algae, also by depressing zooplankton grazers, and (*iii*) benefit small-celled algae because of both reduced grazing and creation of a more nutrient-limited environment that favors smaller cells. Food web models of the type used here provide the only means of assessing the relative importance of these ecological processes.

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Distribution and abundance of stomach nematodes (Anisakidae) among grey seals (*Halichoerus grypus*) and harp seals (*Phoca groenlandica*) in the Gulf of St. Lawrence

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Abstract: In grey seal stomachs, we found in order of decreasing abundance, *Contracaecum osculatum*, *Pseudoterranova decipiens*, *Anisakis simplex*, and *Phocascaris* spp; *Pseudoterranova decipiens* and *C. osculatum* were more abundant than in previous surveys in the Gulf of St. Lawrence. Abundance of *Pseudoterranova decipiens* increased and those of *C. osculatum*, *A. simplex*, and *Phocascaris* spp. decreased along a north–south gradient in the Gulf. Adults were common among *Pseudoterranova decipiens* and *Phocascaris* spp. and rare among *C. osculatum* and *A. simplex*. Among grey seals from Anticosti Island, abundances of *Pseudoterranova decipiens* decreased and those of *C. osculatum* and *Phocascaris* spp. increased between 1988 and 1992. Abundances of *Pseudoterranova decipiens* and *C. osculatum* increased with host age in 1988 and 1992. There was no evidence of displacement of *Pseudoterranova decipiens* by *C. osculatum*. The same four parasites occurred in harp seals (*Phoca groenlandica*), with *C. osculatum* showing an increase compared with earlier surveys. Abundance and prevalence of *Pseudoterranova decipiens* were low (3.4 and 33%, respectively) in harp seals, with few mature individuals. Harp seals are not considered important hosts for *Pseudoterranova decipiens* in this area. Temporal and spatial changes in abundance of *Pseudoterranova decipiens* and *C. osculatum* in the Gulf of St. Lawrence are attributed to an increase in the size of seal populations and a concomitant decrease in mean water temperature.

Résumé : Nous avons identifié en l'estomac des phoques gris par ordre décroissant d'abondance, *Contracaecum osculatum*, *Pseudoterranova decipiens*, *Anisakis simplex* et *Phocascaris* spp. *Pseudoterranova decipiens* et *C. osculatum* étaient tous deux plus nombreux que lors d'études anterieures dans le golfe du St-Laurent, et *C. osculatum* ainsi qu'*A. simplex* etaient plus nombreux dans le nord du golfe qu'ailleurs dans l'est du Canada. L'abondance de *Pseudoterranova decipiens* augmentait, tandis que celles de *C. osculatum*, d'*A. simplex* et de *Phocascaris* spp. diminuaient selon un axe nord–sud dans le golfe. La presence de nematodes adultes était fréquente chez *Pseudoterranova decipiens* et *Phocascaris* spp. et rare chez *C. osculatum* et *A. simplex*. Chez les phoques de l'Ile d'Anticosti, l'abondance de *Pseudoterranova decipiens* a diminué alors que celles de *C. osculatum* et de *Phocascaris* spp. ont augmenté entre 1988 et 1992. Les valeurs d'abondance de *Pseudoterranova decipiens* et de *C. osculatum* augmentaient avec l'âge des phoques en 1988 et en 1992. Il n'y a pas d'évidence de deplacement de *Pseudoterranova decipiens* par *C. osculatum*. Les quatre mêmes parasites étaient présents chez les phoques du Groenland (*Phoca groenlandica*), *C. osculatum* démontrant une augmentation de son abondance depuis les relevés précédents. L'abondance et la prévalence de *Pseudoterranova decipiens* sont faibles (3,4 et 33%, respectivement) et peu sont matures. Le phoque du Groenland n'est pas considéré comme un hôte important pour *Pseudoterranova decipiens* dans cette région. Les variations temporelles et spatiales en abondance de *Pseudoterranova decipiens* et de *C. osculatum* dans le golfe du Saint-Laurent sont attribuées à une augmentation des populations des phoques et, simultanément, à une dimunition de la température moyenne de l'eau.

Introduction

Recent studies have demonstrated that certain species of groundfish are heavily infected with larval sealworm, *Pseudoterranova decipiens* (Ascaridoidea: Anisakidae), and other larval anisakid nematodes in the Gulf of St. Lawrence, eastern Canada (McClelland et al. 1983, 1985, 1987; Boily and Marcogliese 1995; Marcogliese 1995). The sealworm is found in the flesh of commercially important groundfish such as Atlantic cod, *Gadus morhua*, and must be removed manually before fish go to market, resulting in high costs associated with parasite detection and removal, reduced yield, and inferior product (Malouf 1986).

Definitive hosts are seals, the most important of which is the grey seal (*Halichoerus grypus*) (Mansfield and Beck 1977; McClelland 1980*a*). In addition to *Pseudoterranova decipiens*, seal stomachs are infected with other anisakids, including *Contracaecum osculatum*, *Anisakis simplex*, and *Phocascaris* spp., whose larvae also are found in groundfish (Scott and Fisher 1958; Brattey and Ni 1992; Brattey and Stenson 1993).

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Table 1. Summary infection statistics for anisakine nematodes from the stomachs ($n = 286$) of grey seals (*Halichoerus grypus*) collected in the Gulf of St. Lawrence between 1988 and 1992.

	Pseudoterranova decipiens	*Contracaecum osculatum*	*Anisakis simplex*	*Phocascaris* spp.
Prevalence (%)	98.6	99.3	80.8	79.0
Abundance	218.8	2478.3	86.2	32.7
Variance/mean	821.6	3798.9	243.8	54.4
Range of infection	0–4583	0 – 26 528	0–1253	0–318
% adult males	11.4	1.3	0.7	21.7
% adult females	11.6	1.2	0.5	21.3
% larvae	76.9	97.5	98.7	56.9

The grey seal population has increased substantially in the Gulf since the 1960s, from 16 000 – 22 000 seals in 1966 to 42 000 – 67 000 seals in 1987 (Zwanenburg and Bowen 1990). In this study, we examine the geographic distribution of anisakid nematodes among grey seals in the Gulf of St. Lawrence and compare parasite abundances with those obtained during previous surveys in the 1950s and 1970s (Scott and Fisher 1958; McClelland 1980*a*). Furthermore, we examine variations in abundance between years and among age-classes within selected localities. We also test for potential interactions between two putative competitors, *Pseudoterranova decipiens* and *C. osculatum*, as it has been inferred that the latter parasite can displace the former in seal stomachs (McClelland et al. 1985).

The harp seal (*Phoca groenlandica*) is the most numerous seal in the Gulf of St. Lawrence, with an estimated eastern Canadian population of 4.8 million animals (Stenson 1995). Harp seals migrate into the Gulf of St Lawrence and pup on the pack ice near the Magdalen Islands in March. They moult during April and return to the Arctic in late May (Sergeant 1991).

Harp seals from northeastern Newfoundland and Labrador are lightly infected with sealworm (Brattey and Ni 1992), and few sealworm are found in groundfish from this area (McClelland et al. 1987; Brattey et al. 1990). The fact that harp seals also are lightly infected in the Gulf of St. Lawrence, where larval sealworm levels in groundfish are high (McClelland et al. 1983, 1985, 1987; Boily and Marcogliese 1995; Marcogliese 1995) seems to indicate that harp seals are not important final hosts. Mansfield and Beck (1977) estimated that harp seals contribute 18% of the sealworm burden found in fish in the southern Gulf of St. Lawrence, but their estimates are based on the total number of sealworms in harp seals, ignoring the fact that few mature worms were found. To provide a better understanding of their role in transmitting parasites to groundfish, infection levels of sealworm and other anisakid nematodes in harp seals collected from the Magdalen Islands during winter in 1989 and 1991 are compared with earlier surveys from the same area (Myers 1957; Scott and Fisher 1958) and with recent surveys in other areas of eastern Canada (Brattey and Ni 1992).

Materials and methods

Seal stomachs were obtained in the Gulf of St. Lawrence and St. Lawrence estuary by professional seal hunters and research staff. The Gulf of St. Lawrence is divided into northern and southern regions by the Laurentian Channel. Grey seals were collected from Anticosti Island (49°30′N, 63°00′W) in May–June 1988 ($n = 59$) and August–September 1992 ($n = 155$), Les Escoumins (47°21′N, 69°24′W) in August–September 1988 ($n = 17$) and October 1990 ($n = 11$), the Magdalen Islands (47°30′N, 61°40′W) in August–December 1992 ($n = 21$), Port Hood (46°02′N, 61°32′W) in February 1992 ($n = 9$), and Amet Island (45°50′N, 63°11′W) in January 1989 ($n = 8$) and September 1990 ($n = 3$). Harp seals were collected from the Magdalen Islands whelping colony in March 1989 ($n = 37$) and 1991 ($n = 17$). In most cases, seals were weighed and measured, and their jaws removed for subsequent aging. Stomachs were removed, labelled, bagged, and frozen. After thawing, worms were removed and placed in hot 10% glycerol in 70% ethanol to be straightened, fixed, and stored. Nematodes were cleared gradually by evaporation to glycerol and identified to taxon, stage (larva and adult), and, for adults, sex, using a stereomicroscope.

For all statistical analyses, the number of worms of a given species in each seal was transformed ($\log_{10}(n + 1)$). Grey seals were divided into the following age-classes: pups (<1 year), immature (1–5 years), young adults (6–10 years), and old adults (>10 years). For each parasite species in the Anticosti samples, abundance was compared between 1988 and 1992 and among age-classes using a two-way type III analysis of variance (ANOVA) (PROC GLM; SAS Institute Inc. 1988). The analysis was repeated by age-class because of the heterogeneity in sample characteristics. Mass and ages of grey seals in that sample were compared between years using ANOVA (PROC GLM; SAS Institute Inc. 1988). Variations in the proportions of adult sealworm out of the total number of sealworm in each grey seal were tested against the total number of sealworm per seal, the total number of *C. osculatum* per seal, and the grey seal age-class using data from seals with mixed infections of the two parasites, with three-way type III ANOVA (PROC GLM; SAS Institute Inc. 1988). Prior to analysis, the proportions of adult sealworm were transformed using the arcsine transformation (Zar 1984). Correlations between abundances of sealworm and *C. osculatum* (both $\log_{10}(n + 1)$ transformed) by age-class in grey seals containing mixed infections were examined using Pearson product–moment correlation procedures for all seals and for seals according to locality. For statistical analysis of harp seal parasite data, parameters tested were log transformed to homogenize variances and normalize distributions, and then compared between years or among ages using ANOVA (PROC GLM; SAS Institute Inc. 1988). Significance was defined as $p < 0.05$. Terminology conforms to definitions outlined in Margolis et al. (1982).

Results

Grey seals

Nematode species found in grey seal stomachs from the Gulf of St. Lawrence included *Pseudoterranova decipiens, C. osculatum, A. simplex*, and *Phocascaris* spp. *Contracaecum osculatum* was most common, followed in decreasing order by *Pseudoterranova decipiens, A. simplex*, and *Phocascaris* spp. (Table 1). Relatively higher proportions of *Pseudoterranova*

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Table 2. Prevalence (*P*) and abundance (*A*) (mean ± SD) of anisakine nematodes from the stomach of grey seals (*Halichoerus grypus*) collected from various localities in the Gulf of St. Lawrence between 1988 and 1992.

Location	*n*	Mean age (years)	Mean weight (kg)	Date	*P. decipiens*		*C. osculatum*		*A. simplex*		*Phocascaris* spp.	
					P (%)	*A*	*P* (%)	*A*	*P* (%)	*A*	*P* (%)	*A*
Anticosti Island	59	9.3	148.7	May–July 1988	100	300.9±335.1	100	1981.0±2226.8	88.1	117.1±192.1	49.2	8.1±17.2
	155	3.8	99.7	Aug.–Sept. 1992	98.1	102.3±167.6	100	3246.8±3612.6	92.9	100.4±145.9	96.8	47.9±45.2
Les Escoumins	17	4.1	nd	Aug.–Sept. 1988	100	82.1±174.9	100	2097.2±1225.1	70.6	53.6±81.1	70.6	36.4±50.2
	11	2.4	nd	Oct. 1990	100	177.7±189.0	100	1275.2±1284.9	54.5	35.8±38.9	90.9	18.4±14.0
Magdalen Islands	21	4.4	nd	Aug.–Dec. 1992	95.2	288.5±312.2	100	1368.2±2075.9	61.9	34.2±68.1	71.4	26.1±43.1
Port Hood	9	12.8	206.4	Feb. 1992	100	593.2 ±695.5	100	344.2±678.7	11.1	0.1±0.3	66.7	6.4±6.6
Amet Island	8	12.8	nd	Jan. 1989	100	1012.5±856.6	75.0	420.5±848.3	12.5	0.4±1.1	25.0	0.4±0.7
	3	nd	131.7	Sept. 1990	100	1908.3±2319.8	100	27.0±39.0	33.3	0.7±1.2	33.3	0.3±0.6

Note: Collection locations are arranged from north to south. nd, no data available.

decipiens and *Phocascaris* spp. than *C. osculatum* or *A. simplex*, were adults (Table 1).

Prevalence of *Pseudoterranova decipiens* was 95.2–100% at every locality in the Gulf of St. Lawrence, while abundance ranged from 82.1 at Les Escoumins in 1988 to 1908.3 on Amet Island in 1990 and generally increased from north to south (Table 2). Prevalence of *C. osculatum* was 100% in every locality except Amet Island (75%) in 1989. Abundance of *C. osculatum* ranged from 27.0 on Amet Island in 1990 to 3246.8 on Anticosti Island in 1992, and generally decreased from north to south (Table 2). Prevalence of *A. simplex* ranged from 11.1 to 92.9% and abundance, from 0.1 to 117.1; both variables decreased from north to south (Table 2). Prevalence of *Phocascaris* spp. ranged from 25 to 96.8%, and abundance, from 0.3 to 47.9; both variables decreased from north to south (Table 2).

Between 1988 and 1992, abundance of *Pseudoterranova decipiens* decreased at Anticosti Island, while that of *C. osculatum* and *Phocascaris* spp. increased. At Les Escoumins, abundance of *Pseudoterranova decipiens* increased, and that of each of the other nematode species decreased between 1988 and 1990. In the southern Gulf, abundance of *Pseudoterranova decipiens* increased and that of *C. osculatum* decreased at Amet Island between 1989 and 1990, but in seals from Port Hood in 1992, abundance of *Pseudoterranova decipiens* decreased, and that of *C. osculatum* increased compared with nearby Amet Island. Abundance of *Phocascaris* spp. also increased at Port Hood in 1992 compared with Amet Island in 1989–1990. Abundances of the two most common parasites, *Pseudoterranova decipiens* (Fig. 1A) and *C. osculatum* (Fig. 1B), on Anticosti Island generally increased with age in both 1988 and 1992. Within each age-class, abundance of *Pseudoterranova decipiens* decreased (Fig. 1A) and that of *C. osculatum* increased (Fig. 1B) between 1988 and 1992 in grey seals from Anticosti Island.

Analyses of temporal differences and variations in abundance with age-class were confined to Anticosti Island, because sample sizes were small at other localities. The age and mass of seals sampled at Anticosti Island differed between 1988 and 1992 ($p < 0.0001$), the mean age being 9.3 and 3.8 years and mean mass, 148.7 and 99.7 kg in 1988 and 1992, respectively.

Models testing the variation in abundance of the four parasite species in Anticosti grey seals with age-class and year were significant for *Pseudoterranova decipiens* ($p < 0.0001$), *C. osculatum* ($p = 0.0087$), *A. simplex* ($p = 0.0251$), and *Phocascaris* spp. ($p < 0.0001$). Abundance of *Pseudoterranova decipiens* differed among age-classes ($p < 0.0001$) and between years ($p < 0.0001$), but the interaction between age-class and year was not significant. Abundance of *C. osculatum* varied significantly between years ($p = 0.0002$), but not with age-class or the interaction between age-class and year. Abundance of *A. simplex* varied among age-classes ($p = 0.0259$), but not between years or with the interaction between age-class and year. Abundance of *Phocascaris* spp. varied among age-classes ($p = 0.0051$) and between years ($p < 0.0001$), but not with the interaction between age-class and year.

Within each age-class among Anticosti grey seals, abundance of *Pseudoterranova decipiens* differed between 1988 and 1992 for young adults ($p = 0.0050$) and old adults ($p = 0.0098$) but not for pups or immatures. Abundance of *C. osculatum* differed between 1988 and 1992 for pups ($p = 0.0008$) and old adults ($p = 0.0352$), but not for immatures or young adults. Abundance of *Phocascaris* spp. differed between 1988 and 1992 for pups ($p = 0.0005$), immatures ($p \leq 0.0001$), young adults ($p = 0.0003$), and old adults ($p \leq 0.0001$). Abundance of *A. simplex* did not differ significantly between 1988 and 1992 for any age-class.

Abundance of *Pseudoterranova decipiens* was negatively correlated with that of *C. osculatum* in all grey seals infected with both species in the Gulf of St. Lawrence ($r_p = -0.2038$, $p = 0.0009$). When samples were broken down by age-class, site, and also by year for Anticosti, there were no significant correlations between the abundance of *Pseudoterranova decipiens* and that of *C. osculatum*, with the exception of pups from the Magdalen Islands ($r_p = -0.8098$, $p = 0.0014$). Among seals infected with both parasites, the model testing the interaction among the proportion of adult *Pseudoterranova decipiens* relative to the total number of *Pseudoterranova decipiens*, the total number of *C. osculatum*, and host age-class was significant ($p = 0.0007$). The proportion of adult *Pseudoterranova decipiens* varied with age-class ($p = 0.0133$), the interaction between age-class and number of *C. osculatum* ($p = 0.0055$), the interaction between age-class and the number of *Pseudoterranova decipiens* ($p = 0.0094$), and the interaction between age-class, the number of *Pseudoterranova decipiens*,

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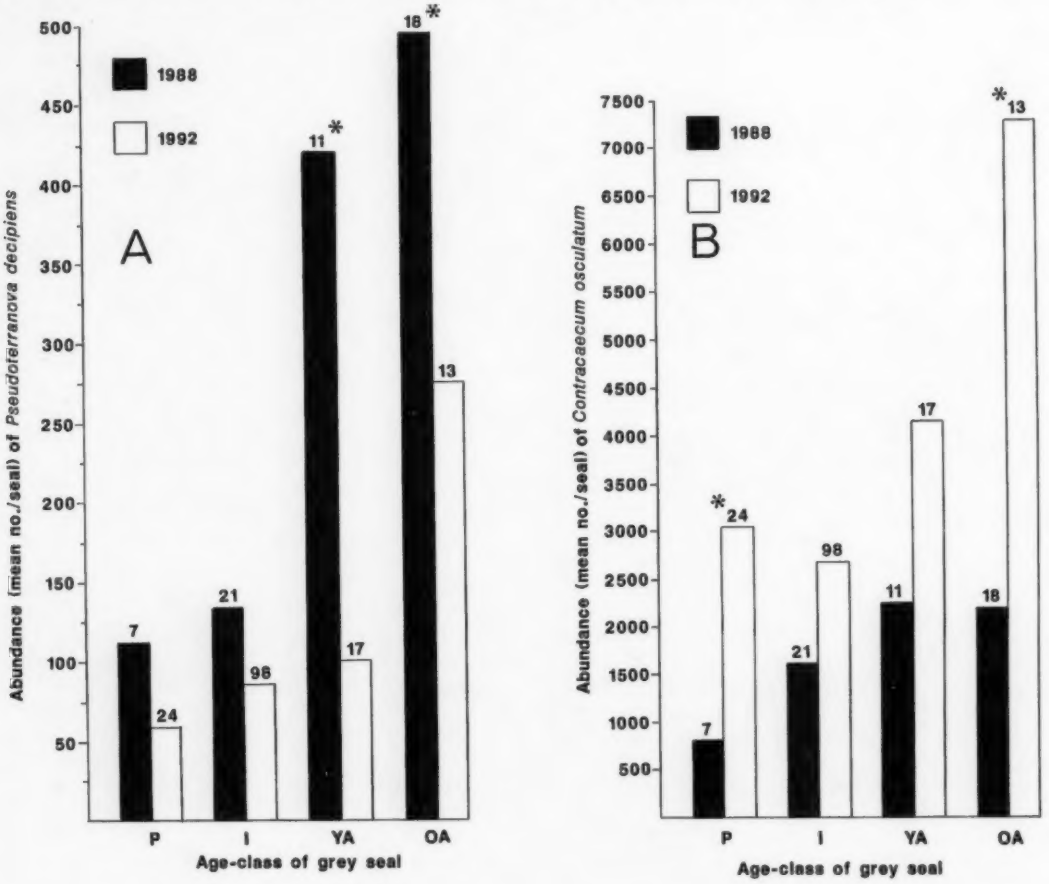
Fig. 1. Abundance of (A) *Pseudoterranova decipiens* and (B) *Contracaecum osculatum* in grey seals from Anticosti Island by age-class in 1988 and 1992. Age-classes are pups (P; <1 year), immatures (I; 1–5 years), young adults (YA; 6–10 years), and old adults (OA; >10 years). Sample sizes are indicated by numbers above each histogram. An asterisk indicates significant differences between years.



and the number of *C. osculatum* ($p = 0.0045$). The proportion of adult *Pseudoterranova decipiens* did not vary significantly with the total number of *Pseudoterranova decipiens* or that of *C. osculatum*, or the interaction between the two. Among seals from Anticosti Island, the proportion of adult *Pseudoterranova decipiens* increased with age (Fig. 2).

Harp seals

Mean masses of seals collected in 1989 (114.7 kg) and 1991 (117.9 kg) were similar. Mean age of seals in 1989 was 10.4 years. Parasite abundance did not vary with age of host in 1989 (no age data available for 1991). There were no significant differences in abundances of *Pseudoterranova decipiens*, *C. osculatum*, *Phocascaris* spp., and *A. simplex* between 1989 and 1991. Therefore, data were combined and summarized (Table 3).

Contracaecum osculatum was the most common nematode found in the harp seal stomachs, with all seals infected at an abundance of 1787 worms per seal. The maximum number of worms in an individual was 4677. Almost 5% of *C. osculatum* were adults (Table 3). Abundance was 1610.1 in 1989 and 2171.0 in 1991. *Pseudoterranova decipiens* was not common in harp seal stomachs, prevalence being 33% and abundance, 3.4 (Table 3). The maximum number of worms occurring in a single host was 71. Almost all sealworm (99.5%) were larvae (Table 3). Prevalence and abundance of sealworm were greater in 1989 (46% and 4.3) than in 1991 (6% and 1.5). *Phocascaris* spp. was not common or abundant, but over 60% of the worms found were adults (Table 3). Prevalence and abundance were similar in 1989 (16% and 1.4) and 1991 (24% and 1.2). *Anisakis simplex* was the least common nematode, occurring in less than 10% of the seals at an abundance of 0.1. No adult worms were found (Table 3). Prevalence and abundance in 1989 were 14% and 0.2, and no worms were found in 1991.

Discussion

Recently, both *Pseudoterranova decipiens* and *C. osculatum* were shown to be species complexes, each with three sibling species in the North Atlantic (Paggi et al. 1991; Nascetti et al. 1993). Only *Pseudoterranova decipiens* B has been found

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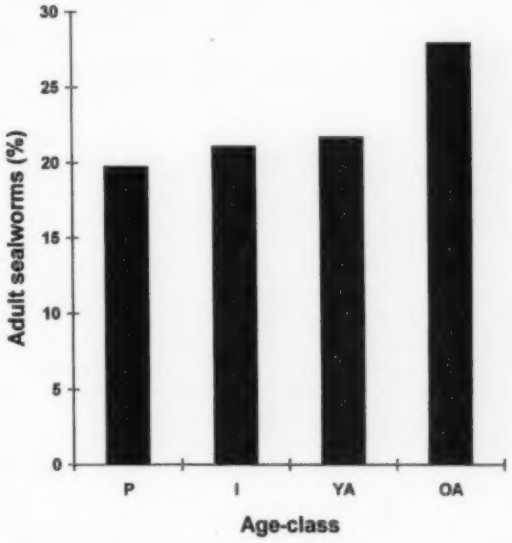
south of Labrador in the northwestern Atlantic Ocean (Paggi et al. 1991; Brattey and Stenson 1993), while both *C. osculatum* A and B were found in the same waters (Brattey and Stenson 1993; Nascetti et al. 1993). However, *C. osculatum* A occurs mainly in bearded seals (*Erignathus barbatus*), while B is found mainly in harp seals but also commonly in grey seals (Brattey and Stenson 1993; Nascetti et al. 1993). Moreover, bearded seals are rare in the Gulf of St. Lawrence. Thus, it is assumed that our species correspond to *Pseudoterranova decipiens* B and *C. osculatum* B. In the northwest Atlantic, *Phocascaris cystophorae* is the most common member of the genus infecting grey seals, but *Phoscascaris phocae* also occurs, as do recombinants of these two species (Paggi and Bullini 1994). Thus, while most of our specimens probably belong to *Phocascaris cystophorae*, we refer to them collectively as *Phocascaris* spp.

The numbers of sealworm and total number of anisakids combined per seal were much greater in grey seals in 1988–1992 in the Gulf of St. Lawrence than in previous surveys. Mean numbers of nematodes found in surveys of adult grey seals between 1948 and 1956 were 158 in the Magdalen Islands ($n = 23$), 700 on Amet Island ($n = 3$), and 496 for the Miramichi area ($n = 69$) (Scott and Fisher 1958). These values are much lower than the total mean number of nematodes per seal from any locality in the Gulf in 1988–1992. A survey of 14 grey seals from Amet Island in 1975–1978 recorded a mean of 528 sealworm per seal (McClelland 1980*a*), compared with >1000 in 1989–1990. Increases in abundance of anisakid nematodes since the 1950s are probably a result of increases in seal populations over the last few decades (Zwanenburg and Bowen 1990; Stenson et al. 1993). Prevalence and abundance of *Pseudoterranova decipiens* from grey seals in the northern localities within the Gulf of St. Lawrence are comparable with those observed from waters around Newfoundland and Labrador in 1985–1993 (Brattey and Stenson 1993), but lower than those in eastern Nova Scotia waters in 1975–1978 (McClelland 1980*b*) and in 1983–1984 (Stobo et al. 1990).

Levels of sealworm infection in harp seals from the southern Gulf of St. Lawrence in 1989 and 1991 were similar (<10) to those recorded from the Magdalen Islands in 1949, 1954, and 1956 (Myers 1957; Scott and Fisher 1958) but were much higher than those reported in harp seals from Newfoundland and Labrador in 1984–1988 (Brattey and Ni 1992). Harp seals may be may be lightly infected with *Pseudoterranova decipiens* as a consequence of their diet, which consists mainly of pelagic crustaceans and fish, such as herring (*Clupea harengus*) and capelin (*Mallotus villosus*) (Myers 1959; Beck et al. 1993), that are seldom infected with sealworm (McGladdery and Burt 1985; Arthur et al. 1995; Marcogliese 1995).

Relatively high proportions of sealworm infections in grey seals consisted of adult worms, whereas few adult sealworm occurred in harp seals from the Gulf of St. Lawrence, and only about 10% of sealworm from harbour seals in the Gulf were adults (D.J. Marcogliese, unpublished data). These observations reaffirm the conclusion of Mansfield and Beck (1977) and McClelland (1980*a*) that grey seals are the most important definitive host for *Pseudoterranova decipiens*. Adult sealworm also were encountered very rarely in harp seal stomachs off Newfoundland and Labrador (Brattey and Ni 1992). Thus, even though the harp seal is the most numerous species in the Gulf of St. Lawrence, with an estimated pup production of

Fig. 2. Proportion, expressed as percentage, of *Pseudoterranova decipiens* occurring as adults in different age-classes of grey seals from Anticosti Island in 1988 and 1992. P, pups ($n = 29$); I, immatures ($n = 118$); YA, young adults ($n = 28$); OA, old adults ($n = 31$).



106 000 near the Magdalen Islands in 1990 (Stenson et al. 1993), it does not appear to be important in sealworm transmission, but the parasite, especially mature worms, may be more numerous in postwhelping seals (Scott and Fisher 1958).

The abundance of *C. osculatum* in grey seals is much higher in the northern Gulf than in waters around Newfoundland and Labrador in 1985–1993 (Brattey and Stenson 1993) and off eastern Nova Scotia in 1975–1978 (McClelland 1980*b*). The highest intensities of *C. osculatum* in the northern hemisphere occurred in grey seals from the northern Gulf of St. Lawrence (see Klöser et al. 1992). Although the abundance and prevalence of *C. osculatum* were very high in grey and harp seals, few adults occurred in grey seals in comparison with harp seals. Thus, while grey seals are capable of sustaining heavy infections of this parasite, they may not be important for its transmission in the Gulf of St. Lawrence. The abundance in harp seals reported herein is much higher than that reported in those from the Magdalen Islands in 1956 (Myers 1957) and that reported for all nematodes combined (other than sealworm) from the same areas in the 1950s (Scott and Fisher 1958). Contracaecine larvae also have become increasingly abundant in cod throughout the Gulf of St. Lawrence in recent years (Boily and Marcogliese 1995). Seals and cod may acquire the parasite by eating capelin, which are heavily infected with contracaecine larvae in the Gulf of St. Lawrence (Arthur et al. 1995; Marcogliese 1995). Increasing abundance of *C. osculatum* may be attributable to the growth of the harp seal population (Stenson et al. 1993). While *C. osculatum* abundance in harp seals examined from Newfoundland and Labrador was much lower (Brattey and Ni 1992) than that found in harp seals herein, the proportion of mature worms in seals from the former was consistently higher. Our samples, however, were collected during the breeding season, and as McClelland

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Table 3. Summary statistics for anisakid nematodes in the stomachs of harp seals, *Phoca groenlandica*, collected from the Magdalen Islands in the Gulf of St. Lawrence in March 1989 and 1991.

Nematode species	*P* (%)	*A*	Variance/mean	Range	% adult males	% adult females	% larvae
Anisakis simplex	9.3	0.1	1.2	0–2	0.0	0.0	100
Contracaecum osculatum	100	1786.7	793.2	22–4677	2.5	2.3	95.2
Phocascaris spp.	18.5	1.3	12.8	0–20	20.0	41.4	38.6
Pseudoterranova decipiens	33.3	3.4	38.8	0–71	0.0	0.5	99.5

Note: Sample size was 54 seals. *P*, prevalence; *A*, abundance.

(1980*a*) noted, adult sealworm from breeding or moulting seals are usually small and flaccid, and worms may be shed during the associated fasting period.

Few adults of *A. simplex* were observed in Gulf grey and harp seals, and cetaceans are the normal definitive host (van Thiel 1966). Levels in grey seals from the northern Gulf are higher than in those in other parts of eastern Canada (McClelland 1980*b*; Brattey and Stenson 1993). Cetaceans are very abundant in the northern Gulf of St. Lawrence during ice-free periods, thus accounting for the distribution of *A. simplex* in both grey seals and fish (Boily and Marcogliese 1995; Marcogliese 1995). Seals acquire infections by feeding on herring and capelin, which are heavily infected in the Gulf of St. Lawrence (McGladdery and Burt 1985; Arthur et al. 1995; Marcogliese 1995).

Levels of *Phocascaris* spp. in both grey and harp seals are substantially lower than those reported by Brattey and Ni (1992) for Newfoundland and Labrador in 1984–1988. Almost half of *Phocascaris* spp. were adults in the Gulf grey seals, with 61% mature in harp seals, suggesting that both host species may be important hosts. Abundance of this parasite was low in all localities, but it is difficult to reach conclusions regarding this nematode because it also occupies the anterior portion of the small intestine (Brattey and Stenson 1993), which was not examined in this study. However, given the low numbers observed in the stomachs of either seal species, it may be relatively uncommon in the Gulf of St. Lawrence, especially in southern waters.

The abundance of the two most common nematodes, *Pseudoterranova decipiens* and *C. osculatum*, increased with grey seal age, although only significantly so for the former. These results are similar to those of Bjorge (1984) and Stobo et al. (1990) and are probably related to increased prey consumption or the ingestion of larger more heavily infected fish by older, larger seals.

Results herein indicate that abundance of *Pseudoterranova decipiens* is decreasing, while that of *C. osculatum* is increasing, in grey seals in the Gulf. These patterns are similar to those observed in Atlantic cod in 1990–1992 in the Gulf (Boily and Marcogliese 1995). Abundance of sealworm also decreased in white hake (*Urophycis tenuis*) and longhorn sculpin (*Myoxocephalus octodecemspinosus*) during the same period (Marcogliese 1995). It is difficult to discriminate cause and effect from these observations. The decrease in abundance of sealworm in cod and other groundfish in the Gulf of St. Lawrence may result from a corresponding decrease in abundance of this parasite in grey seals, the most important definitive host. A simultaneous increase in contracaecines in cod may result from an increase in the harp seal population (Stenson et al. 1993), harp seal being the most important definitive host for *C. osculatum*. However, these hypotheses do not explain the cause of the decline of the sealworm population in grey seals between 1988 and 1992.

The observed changes in parasite abundances between 1988 and 1992 may be due to size differences between age-classes, especially as adult males grow much larger than adult female grey seals (McLaren 1993). Indeed, in 1988 the old adults (>10 years) were mainly large males, whereas in 1992 that age group consisted primarily of smaller females. This difference possibly could explain the decrease observed in sealworm abundance within this age group, but not the increase observed in abundance of *C. osculatum* in the same group. Furthermore, the decrease in sealworm abundance among young adults and increase of *C. osculatum* among pups cannot be explained by size differences between 1988 and 1992.

Changes in diet may account for changes in abundance of stomach nematodes in grey seals (Wiig 1988; Stobo et al. 1990; Haug et al. 1991). Grey seals consume both demersal and pelagic prey (Bowen et al. 1993), and their diet in the Gulf of St. Lawence consists primarily of herring, cod, and capelin (Benoit and Bowen 1990; Murie and Lavigne 1992). Among fish hosts, the sealworm occurs primarily in demersal fishes (McClelland et al. 1990), whereas contracaecines occur both in demersal and pelagic fishes, including capelin (Marcogliese 1995). Increased consumption of pelagic fishes in 1992 as compared with 1988 could cause the observed changes in parasite abundances. However, the grey seals collected from Anticosti Island in 1988 fed mainly on capelin, while those in 1992 fed heavily on cod (Proust 1996). Furthermore, an increase in consumption of pelagic fishes would lead to an increase in abundance *A. simplex*, which is found mainly in pelagic hosts (McClelland et al. 1990), yet no differences were found in abundance of this parasite within grey seal age-classes between 1988 and 1992. Thus, alterations in diet cannot explain the increase in abundance of *C. osculatum* and the simultaneous decrease in sealworm abundance observed in grey seals from Anticosti Island between 1988 and 1992.

The abundance of *Pseudoterranova decipiens* and *C. osculatum* in both fish and seals is inversely related, and *C. osculatum* abundance is increasing in cod in the southern Gulf of St. Lawrence (Boily and Marcogliese 1995). It has been suggested that there may be competitive interactions between the two parasite species, with *C. osculatum* displacing *Pseudoterranova decipiens* (McClelland et al. 1985). However, Brattey and Stenson (1993) found no evidence of negative interactions between the two species in seals, nor were any negative associations found within any locality in this study, with a single exception. Furthermore, the proportion of adult sealworm was not affected by the abundance of sealworm nor that of *C. osculatum*. Therefore, development and maturation of sealworm does not appear to be impeded by *C. osculatum*. The overall

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negative correlation between the two parasites from the pooled seal samples is not a consequence of interspecific competition, and inferences from such analyses should be cautioned against.

Abiotic factors, such as temperature, also may be important in determining distribution and abundance of anisakid nematodes (Templeman et al. 1957). During the years 1990–1992, mean bottom temperatures in the cold intermediate layer (30–100 m) in the Gulf of St. Lawrence dropped more than 1°C to 0.31–0.39°C compared with long-term mean temperatures, and this water layer consistently has experienced a drop in mean annual temperature since 1986 (Gilbert et al. 1996). In a British study, grey seals spent the majority of their submerged periods foraging at depths of 30–70 m (Thompson et al. 1991), where they presumably defecate, thus releasing nematode eggs into the environment. While both *C. osculatum* and *Pseudoterranova decipiens* eggs hatch at 1.7°C (Brattey 1990), only eggs of *C. osculatum*, and not those of *Pseudoterranova decipiens*, hatch at 0°C (L. Measures, Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, Que., personal communication). The decrease in bottom temperature observed in the Gulf of St. Lawrence in the 30–100 m depth layer may hinder the development of *Pseudoterranova decipiens*, but not that of *C. osculatum*. Water temperatures also may explain the decrease in abundance of *C. osculatum*, and the increase in that of *Pseudoterranova decipiens*, along a north–south latitudinal gradient in the Gulf as seen in grey seals and cod (Boily and Marcogliese 1995; the present study). The observed changes in the distribution and abundance of these two parasites in the Gulf of St. Lawrence may be caused by a combination of increases in grey and harp seal densities concomitant with climatic changes affecting bottom temperature. We predict that should water temperatures increase, abundance of sealworm will also increase in fish and seals in the Gulf of St. Lawrence.

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Evaluation of gladius dorsal surface structure for age and growth studies of the short-finned squid, *Illex illecebrosus* (Teuthoidea: Ommastrephidae)

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Abstract: The potential use of growth increments deposited on the gladius of the short-finned squid (*Illex illecebrosus*) for age determination and growth studies was investigated. The gladius lies along the dorsal midline of the mantle and grows from the fins towards the head by the addition of layers of chitin. Growth increments were observed on the dorsal surface of the gladius and could be consistently enumerated. The frequency of deposition approximated statolith incremental deposition rates and the daily nature of gladius growth increments during the adult phase was supported. Total age could not be obtained by gladius growth increment counts on the dorsal surface because, as the gladius grows, the posterior zone becomes progressively vague and the early growth increments are masked. However, gladius increments were highly correlated with mantle growth and provided accurate estimates of size-specific growth. Both group and individual growth trajectories were reconstructed. The latter seemed to be sensitive to feeding events, temperature fluctuations, and maturation processes in captive squids. The potential for reconstruction of individual growth histories and for the study of growth patterns in wild populations is discussed.

Résumé : Nous avons examiné le potentiel d'utilisation des couches de croissance déposées sur le gladius de l'encornet nordique *Illex illecebrosus* pour mesurer l'âge et faire des études sur la croissance de cette espèce. Le gladius se trouve le long de la ligne médiane dorsale du manteau et grossit à partir du niveau des nageoires, en direction de la tête, par l'ajout de couches de chitine. Les marques de croissance ont été observées sur la face dorsale du gladius et ont pu être comptées de façon uniforme. La fréquence de dépôt correspondait approximativement à celle des marques de croissance des statolithes, et la nature journalière des dépôts pendant la phase adulte a été confirmée. Il n'a pas été possible d'obtenir l'âge total en comptant les marques de croissance sur la face dorsale du gladius car, à mesure que celui-ci grandit, sa zone postérieure devient de plus en plus floue, et les premières marques de croissance sont masquées. Toutefois, les accroissements du gladius étaient fortement corrélés à la croissance du manteau, et fournissaient des estimations précises de la croissance spécifique à la taille. Nous avons construit le schéma de croissance du groupe et des individus. Ce dernier semblait sensible aux événements d'ordre alimentaire, aux fluctuations de la température et aux processus de maturation chez les encornets captifs. Nous analysons le potentiel de cette méthode pour reconstituer l'histoire de la croissance individuelle et étudier les schémas de la croissance chez les populations sauvages.
[Traduit par la Rédaction]

Introduction

During the past 20 years, significant advances in cephalopod population biology have derived from the development of indirect methods of ageing (reviewed by Jereb et al. 1991). Analogous to fish otoliths, cephalopod statoliths have been shown to provide reliable age estimates in days and have become the main tool for the study of growth, cohort analysis, and life-history reconstruction (Caddy 1991; Jackson 1994; Arkhipkin and Perez 1996). More recently, the potential of another cephalopod skeletal structure, the gladius, for age and growth studies has been investigated (Bizikov 1991; Arkhipkin and Bizikov 1991; Jackson et al. 1993).

The gladius is the internal shell present in squids and sepiolids. Already formed at the moment of hatching, the gladius structure is uncalcified and mainly composed of the polysaccharide β-chitin in association with proteins (Hunt and Nixon 1981). Periodic growth increments were first observed by Naef (1922) and LaRoe (1971). Bizikov (1991) described the inner structure of the gladius and showed that, homologous to the shell of other molluscs, it consisted of a middle shell layer (ostracum), an inner shell layer (hypostracum), and an outer shell layer (periostracum), with different planes of accretive growth potentially suitable for age interpretation.

By comparison with statolith-determined age, preliminary studies have supported the daily deposition of the growth increments on the periostracum and the hypostracum of the squids *Kondakovia longimana* and *Berryteuthis magister*, respectively, suggesting that such growth layers are suitable for age and growth studies (Arkhipkin and Bizikov 1991; Bizikov

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Table 1. Summary of short-finned squid (*Illex illecebrosus*) samples used in the evaluation of the gladius structure for age and growth studies including vessel, cruise date, number of squid (*n*), size range (ML, mantle length), and preservation of the material.

Vessel	Date	*n*	ML range (mm)	Mass range (g)	Preservation
RV *Belogorsky*	Apr. 1979	148	11.8–65.4	0.08–6.23	p
RV *Gadus atlantica*	Feb. 1981	308	11.1–30.1	0.08–0.62	p
RV *Lady Hammond*	Feb. 1982	346	10.6–43.8	0.07–1.81	p
RV *Needler*	Jan. 1985	121	7.8–57.4	0.04–3.48	p
RV *Needler*	Feb. 1988	94	11.0–60.2	0.15–4.51	p
RV *Needler*	Apr. 1989	279	18.7–92.3	0.23–16.11	p
RV *Needler*	June 1989	40	18.8–67.9	0.2–5.57	p
RV *Needler*	Aug. 1989	60	30.6–46.5	0.77–1.97	p
FV *Rio Salado*	June 1991	62	119.0–192.0	30.0–120.0	ff
FV *Rio Damuji*	June 1991	70	118.0–188.0	31.0–100.0	ff
RV *Petrel*	July 1991	51	143.0–201.0	45.0–151.0	ff
Total		1579	7.8–201.0	0.04–151.0	

Note: p, fixed in formalin and preserved in ethanol; ff, fresh frozen.

1991). In the ommastrephids *Illex argentinus* and *Sthenoteuthis oualaniensis* and the loliginid *Sepioteuthis lessoniana*, the periostracum and the hypostracum are not well developed, but growth increments are distinguished directly on the dorsal surface of the ostracum. In these species, ostracum growth increments were found to be deposited with a near-daily periodicity, but total age determination using this layer could not be obtained (Arkhipkin and Bizikov 1991; Bizikov 1991, 1995; Jackson et al. 1993). The ostracum is responsible for longitudinal growth of the gladius and parallels the growth of the mantle. Because mantle length is the standard measure of squid size (Amaratunga and Durward 1978; Roper and Voss 1983), ostracum growth increments may provide accurate size-specific somatic growth rate estimations (Arkhipkin and Bizikov 1991). Size-specific growth has been shown to provide important insights into the growth performances of both groups and individuals, complementing age-derived statolith applications to squid population ecology (Arkhipkin and Perez 1996).

The short-finned squid, *Illex illecebrosus*, sustains a valuable but highly unpredictable seasonal fishery in the northwest Atlantic. Floating egg masses are spawned throughout the year, presumably south of 34°N over the Blake Plateau. Developing eggs are carried north by the Gulf Stream to supply geographical subpopulations with annual recruits. Juveniles enter the coastal waters of the Scotian Shelf and Newfoundland during spring and summer and then migrate south, toward the spawning grounds, with the onset of sexual maturarion (O'Dor 1983). Since the validation of statolith growth increments (Dawe et al. 1985; Hurley et al. 1985), studies on growth and population structure (Dawe et al. 1985; Dawe and Beck 1992) have greatly improved our understanding of the mechanisms of population regulation (O'Dor 1992; O'Dor and Coelho 1993). This work investigates the accretive growth on the dorsal surface of the ostracum of the gladius of *I. illecebrosus* and evaluates its potential for age determination and reconstruction of growth histories.

Throughout the text we use the terms juvenile and adult to differentiate micronektonic individuals caught offshore in the slope waters from fully nektonic squids that occur on the continental shelf and that are caught by commercial trawling, respectively.

Materials and methods

Frozen samples of adult *I. illecebrosus* caught on the Scotian Shelf were obtained from the commercial fishery and from one research cruise of the RV *Petrel* in the summer of 1991. Samples of juvenile squid were collected during eight research cruises conducted in the Gulf Stream – Slope water front (25–44°N) from 1979 to 1989 (Table 1) and preserved in 70% ethanol. One *I. illecebrosus* paralarva (3.8 mm dorsal mantle length (ML)) was obtained from a plankton survey conducted off the coast of Florida (26°30′N, 79°59′W) in January 1986 and was preserved in formalin. Frozen squid were thawed and weighed to the nearest gram, the ML was measured to the nearest millimetre, and statoliths and gladii were extracted and stored in 70% ethanol and formalin, respectively. Only the gladius was extracted from preserved squid. After the measurements were taken on preserved specimens, the mantle was briefly plunged into a solution of NaOH (1 M) at approximately 60°C to soften the tissue and facilitate gladius dissection.

Gladius analysis

The paralarval gladius was examined under a Nanolab 2000 scanning electron microscope. For this analysis, the gladius was mounted on an aluminum stub on double-sided tape, air dried for 24 h, and coated in a vacuum evaporator with 82 Å (1 Å = 0.1 nm) of gold. In juveniles and adults, gladii were prepared and initially read using the relative growth technique, for comparison with the results of Bizikov (1991). Each gladius was measured for total length (GL) and marked at 10-mm intervals from the posterior (fins) to the anterior end (head) (Fig. 1). The dorsal surface of the ostracum was then examined under a Zeiss dissecting microscope at 20–30× using a combination of incident and transmitted light to optimize the visualization of growth increments. Growth increments of the ostracum were counted per gladius length interval from the anterior (head) toward the posterior (fins) end until increments were no longer visible (Fig. 1). The mean spacing between growth increments (mean growth increment in millimetres) was calculated by dividing 10 mm by the number of growth increments counted in a gladius length interval.

The precision of gladius increment counts was assessed by comparing three blind counts of 30 selected squids made independently by a single reader with a 1-month interval between counts. The total numbers of increments counted in a fixed proportion of the gladius length on the three occasions were compared by (*i*) the average percent error (APE; Beamish and Fournier 1981) and (*ii*) the coefficient of variation (CV; Chang 1982).

Gladius growth histories were reconstructed for preserved specimens that had been held in the 15 m diameter pool of the Aquatron Laboratory of Dalhousie University, Halifax, N.S., in 1978 for tagging,

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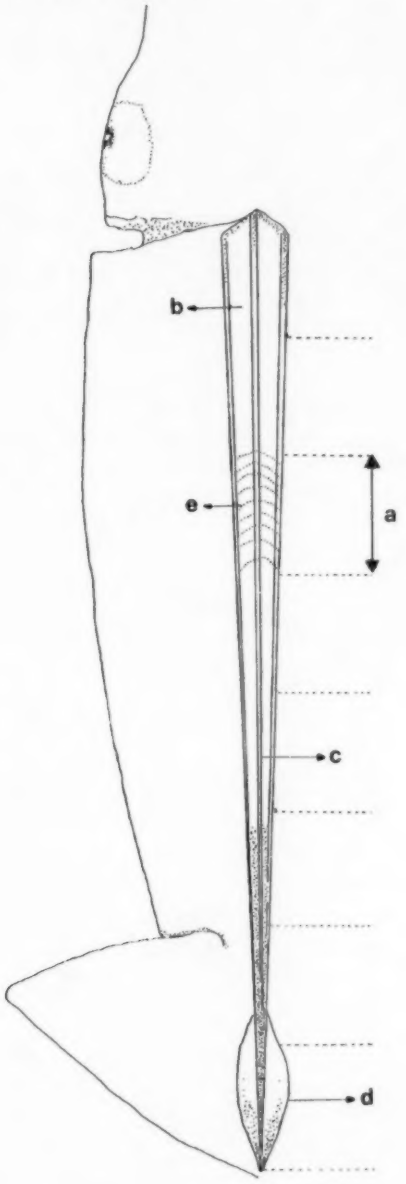
Fig. 1. Schematic dorsal view of *Illex illecebrosus* gladius and position in relation to the squid mantle. *a*, 1-cm interval; *b*, gladius plate; *c*, rachis; *d*, conus; *e*, growth marks. The stippled area corresponds to the portion of the gladius where the growth increments are not distinguishable.



feeding, and growth experiments (O'Dor et al. 1980). Gladii were extracted from three preserved specimens identified by colour-coded tags: male tag red–yellow–orange (RYO), 170 mm ML, 118 g mass; male tag red–green–red (RGR), 195 mm ML, 148 g mass; and female tag red–orange–red (ROR), 235 mm ML, 246 g mass. The squids were captured and tagged on 18 July 1978, and feeding was individually controlled between 19 July and 4 August. After that the individuals were allowed to feed freely (O'Dor et al. 1980). RYO, RGR, and ROR survived 24, 18, and 56 days in the tank, respectively. Series of growth increments were obtained by displaying the image of the gladius dorsal surface on a monitor and measuring distances between consecutive growth increments with an image analysis system (Bioscan OPTIMAS). Each growth series was qualitatively compared with monitored feeding rates and temperature fluctuations. In the female ROR, which survived longer than the other two and experienced significant temperature variations in the tank, a technique of multiple series analysis known as transfer function (Box and Jenkins 1976) was used to represent the magnitude and lag of growth variability (dependent series) in response to temperature fluctuations (independent series). As trends exhibited by either series can generally produce spurious correlation between them, this technique requires a previous detrending of the series. In our case, temperature series presented an increasing trend (see Fig. 8), which was removed by "differencing" the original temperature series.

Statolith analysis

To investigate the rate of deposition of gladius increments, statolith daily rings of 20 selected squids were compared with partial counts of their gladius growth increments. Age was determined on the statolith structure according to the method described in Dawe et al. (1985) and reviewed in Jereb et al. (1991). The right statolith of each squid was mounted on a glass slide and ground in the sagittal plane. Ground surfaces were examined under a light microscope at 200× magnification with a camera lucida and a drawing arm was used to outline the projected growth increments on a paper pad. The total number of growth rings, counted along the axis of maximum growth, was compared with the number of growth increments deposited anterior to the initial 100 mm of the gladius length (as measured from the posterior end) of the same individuals (Fig. 1).

Results

Relationship between gladius and somatic growth

GL was measured in 1576 squid including preserved and fresh-frozen individuals (Table 2). A strong linear correlation was found between ML and GL (Fig. 2A), with ML explaining 99.4% of the total variance of GL (Table 2). A curvilinear model was fitted to the relationship between GL and total wet mass ($r^2 = 0.986$) after log linearization of the variables (Fig. 3A; Table 2).

The residual analysis of both relationships, however, showed that GL of large fresh-frozen squids was usually underestimated by both fitted models (Figs. 2B and 3B). When compared, slopes of regressions calculated separately for preserved and fresh-frozen squids were found to be significantly different (Table 2), indicating a possible preservation artefact in the relationship between GL and somatic growth. It was supposed this artefact derived mostly from the dehydration of the mantle in ethanol, which causes both shrinkage of tissues and loss of mass. To test this, an additional 19 fresh squids were weighed and their ML was measured before and after fixation in formalin and preservation in 70% ethanol for 40 days. A linear relationship obtained between preserved and fresh ML ($r^2 = 0.866$) and mass ($r^2 = 0.983$) provided a factor that was then used to correct the ML and mass of the fresh-frozen squids used in the relationships between GL and somatic growth. The correction for preservation of ML increased the slope of the ML × GL relationship, suggesting that preservation causes shrinkage of the mantle. It is possible, however, that the preservation time of fresh squid for correction of ML

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Table 2. Linear regression of gladius length (GL) on mantle length (ML) and of log GL on log mass (M) of preserved, fresh-frozen, and fresh-frozen "corrected" for preservation specimens.

ML × GL					
	n	ML (mm)	a	b	r^2
Preserved	1393	7.8–92.3	5.235±0.273	1.027±0.008	0.977
Fresh frozen	183	118–201	13.881±4.759	0.874±0.031*	0.946
Corrected	183	118–201	15.458±4.678	0.905±0.031*	0.946
Total	1576	7.8–201.0	8.178±0.208	0.920±0.004	0.994
log M × log GL					
	n	M	log a	b	r^2
Preserved	1393	0.04–16.11	1.593±0.002	0.355±0.004	0.972
Fresh frozen	154	30.0–151.0	1.648±0.026	0.295±0.014*	0.916
Corrected	154	30.0–151.0	1.565±0.026	0.297±0.014*	0.916
Total	1547	0.04–151.0	1.587±0.002	0.339±0.002	0.986

Note: a, intercept; b, slope. Values for a and b are given as the mean ± 95% CI at $\alpha = 0.05$. Asterisks indicate that the slopes calculated for preserved specimens were found to be significantly different from the slopes calculated for fresh-frozen specimens even after correction for the fixative effect.

Fig. 2. Relationship between mantle length and gladius length. (A) Linear regression, GL = 8.18 + 0.92(ML) ($r^2 = 0.994$). (B) Residuals. Vertical bars represent preserved specimens; triangles represent fresh-frozen specimens.

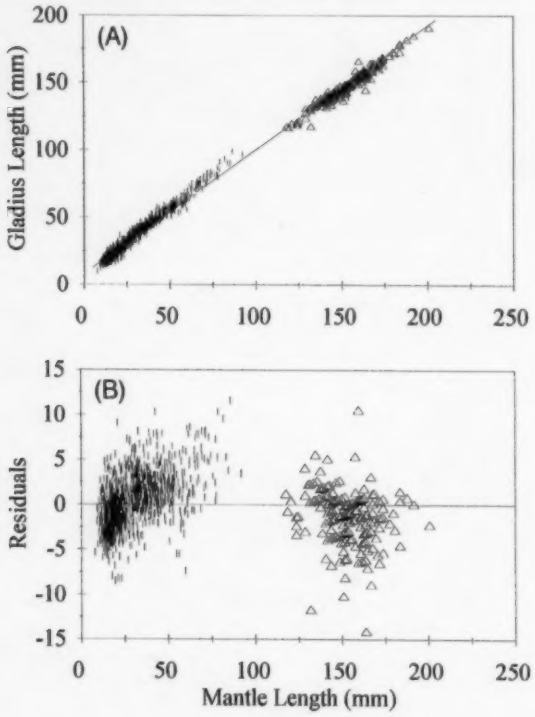
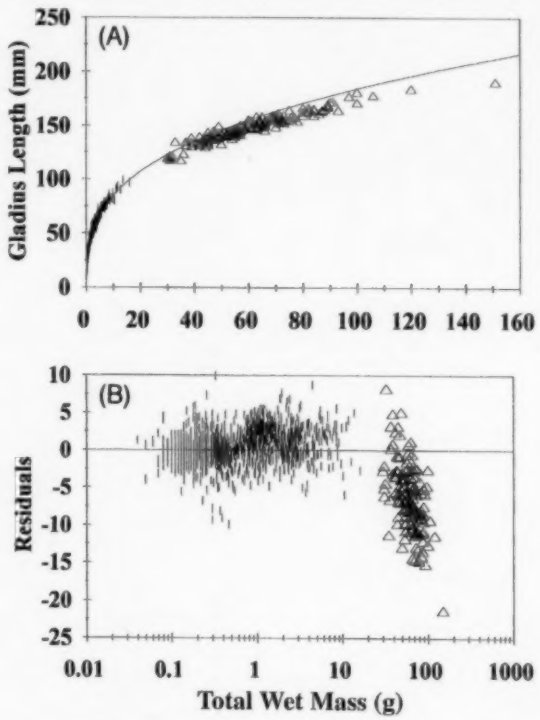


Fig. 3. Relationship between total mass and gladius length. (A) Model fitted after log linearization of the variables, GL = $38.64(ML)^{0.339}$ ($r^2 = 0.986$). (B) Residuals. Vertical bars represent preserved specimens; triangles represent fresh-frozen specimens.



values was not long enough, since after correction, the slopes were still significantly different (Table 2).

Gladius morphology and growth increments

In *I. illecebrosus*, the gladius is typical of a pelagic fast-swimming open-ocean squid. It consists of a narrow central rachis with two lateral ribs and a terminal spoon-shaped conus (Fig. 1). This structure is mostly formed by the growth of the ostracum layer, while the periostracum and the hypostracum layers are poorly developed and inadequate for age and growth interpretation. The longitudinal growth of the gladius occurs by the deposition of chitin layers to the ventral surface of the

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Fig. 4. Accretive growth of the ostracum on the gladius of *Illex illecebrosus*. (A) Deposition of layers on the anterior border (head). Scale bar = 0.1 mm. (B) Growth increments as observed on the dorsal surface of the gladius (triangles). Scale bar = 0.5 mm.

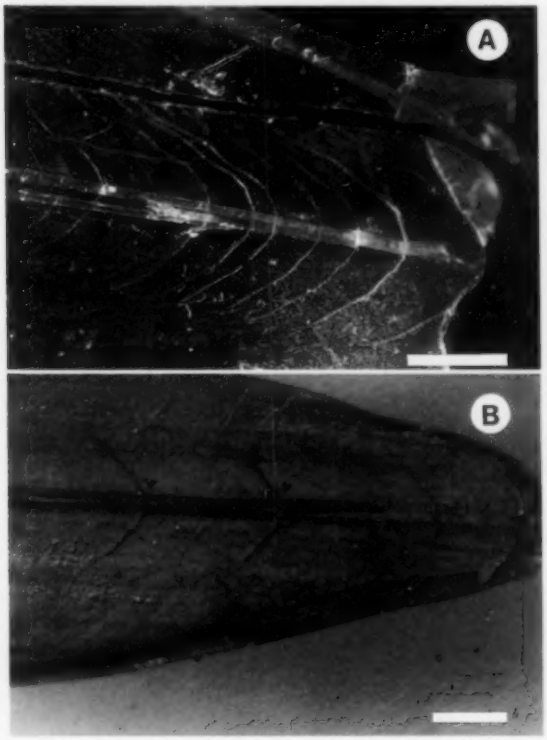
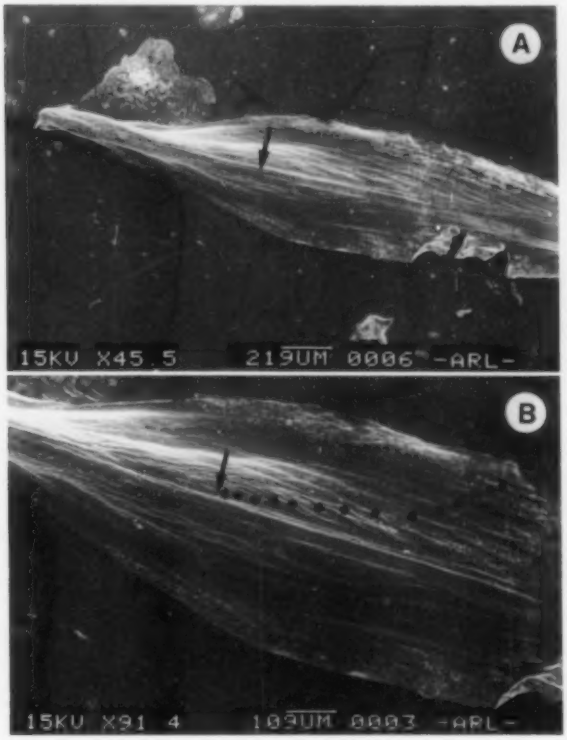


Fig. 5. Scanning electron micrographs of the dorsal gladius structure of an *Illex illecebrosus* paralarva (3.7 mm GL). (A) ×45.5 magnification; (B) ×91.4 magnification. The arrow indicates the estimated GL at hatching. Asterisks indicate the visible growth increments. The anterior end of the gladius (head) is to the right side.



ostracum from the fins towards the head (Figs. 1 and 4A). During periods of slow growth, such layers crowd together and form a distinct discontinuous mark that resembles the anterior border of the gladius (Fig. 4B), the increments observed closer to the border being the ones more recently deposited.

In juveniles and adults, growth increments were observed on the dorsal surface of the rachis (Fig. 4B). These increments became faint as the gladius narrows posteriorly and could not be distinguished near the conus region (Fig. 1). Because early gladius growth was masked, the absolute number of growth increments on the ostracum layer could not be determined.

In the paralarval stage, regularly spaced growth increments were seen directly on the dorsal surface of the broad and relatively longer conus (Fig. 5). The first growth mark, corresponding to the hatchling gladius, was not readily distinguishable (Fig. 5B). From estimations of GL at hatching, however, it is possible that the total number of growth increments counted anterior to this length approximated absolute age. *Illex illecebrosus* specimens hatched in the laboratory measured around 1.10–1.25 mm ML (O'Dor et al. 1986). In Fig. 5B, around 14 increments could be counted between 1.1 mm GL and the anterior border of the conus.

Growth increment interpretation

Gladius growth was analyzed in 343 squids ranging from 11 to 201 mm ML and from 0.2 to 151 g mass. On average, growth increments were visible in the anterior half of the gladius (percentage readable of GL, 49.12 ± 9.75; mean ± SD), but were altogether unreadable in 18 gladii. The number of increments counted per centimetre interval of GL ranged from 7 to 20. The mean spacing between growth increments within a GL interval was more variable and increased from 0.55 to 0.76 mm on average in the earliest deposited 20–50 mm of the GL. In gladii longer than 60 mm, increment variability decreased and the mean spacing stabilized at around 0.91–0.98 mm (Table 3).

Growth increments on the ostracum dorsal surface were consistently enumerated as shown by the analysis of the precision of growth increment counts in a fixed proportion of GLs. The mean APE calculated for the three blind determinations was 3.77% and the mean CV was 5.11%.

The rate of gladius increment deposition was assessed by the relationship between the number of daily statolith increments and gladius growth increments in 20 selected squids (131–180 mm ML, 42–91 g). A linear relationship with a slope of 1 would be expected if gladius growth increments were deposited at the same rate as statolith increments. In addition, because gladius counts for each squid included all increments deposited anterior to 100 mm GL, this assumption predicts that the fitted line should intercept the *y* axis at an age estimate for a 100-mm GL squid. Statolith and gladius growth

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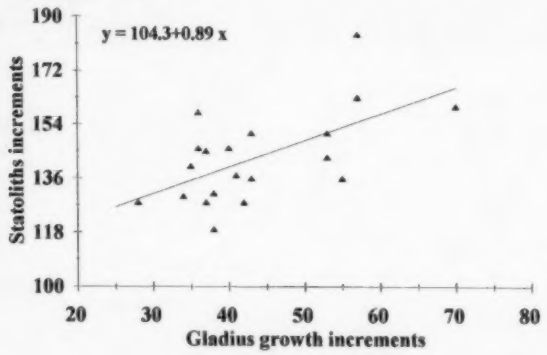
Table 3. Number of growth increments and mean increment width in millimetres (10 mm divided by the number of growth increments) per 10 mm of gladius length intervals.

		Number of increments		Increment width (mm)	
GL (mm)	*n*	Mean±SD	Range	Mean	Range
10–20	45	18.14±1.91	15–20	0.55	0.38–0.83
20–30	53	15.85±1.64	12–20	0.63	0.50–0.83
30–40	44	14.28±1.74	11–18	0.70	0.55–0.91
40–50	37	13.06±1.85	11–17	0.76	0.59–1.00
50–60	26	11.30±0.93	10–14	0.88	0.71–1.11
60–70	78	10.26±0.95	8–13	0.97	0.77–1.25
70–80	122	10.35±1.12	8–13	0.97	0.77–1.25
80–90	158	10.43±1.06	8–13	0.96	0.77–1.25
90–100	178	10.47±1.17	7–14	0.95	0.71–1.43
100–110	181	10.62±1.22	7–14	0.94	0.71–1.43
110–120	179	10.57±1.13	8–14	0.95	0.71–1.25
120–130	173	10.69±1.22	8–14	0.94	0.71–1.25
130–140	139	10.55±1.26	8–14	0.95	0.71–1.25
140–150	85	10.58±1.35	8–14	0.95	0.71–1.25
150–160	38	10.29±1.21	8–13	0.97	0.77–1.25
160–170	11	10.45±0.78	9–12	0.96	0.83–1.11
170–180	5	11.00±0.63	10–12	0.91	0.93–1.00
180–190	1	10.00±0.00	—	—	—
Total	1553		7–20	—	—

Note: *n*, number of GL intervals.

Fig. 6. Relationship between the number of statolith growth increments and the number of gladius growth increments anterior to 100 mm GL. The solid line represents the linear model fitted to the data set ($r^2 = 0.37$).

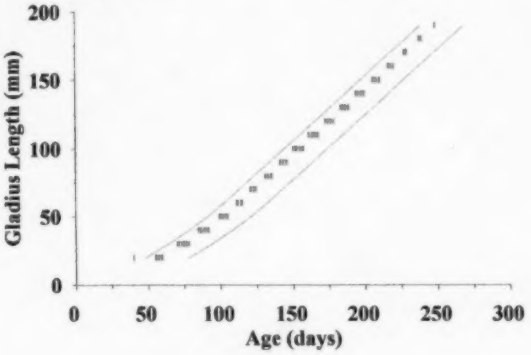


increments correlated weakly (Fig. 6; $r^2 = 0.366$). However, the slope obtained (0.89 ± 0.21, 95% CI) was not significantly different from the hypothesized slope of 1 ($t = 0.414$, $p > 0.5$; Fig. 6), and a total of 104.3 ± 25.9 (95% CI) statolith increments was estimated by the model for 100 mm GL. The number of statolith increments estimated for *Illex* spp. of 100 mm ML, reported by other studies, varies between 115 and 120 (Hurley and Beck 1979; Morris and Aldrich 1985; Arkhipkin 1990).

Reconstruction of mean growth trajectory

A gladius growth curve was reconstructed from the cumulative number of growth increments counted in each centimetre

Fig. 7. Growth curves reconstructed from gladius analysis. Age at sizes were estimated by the cumulative number of growth increments counted in 10-mm GL intervals. The curves were adjusted assuming statolith age estimations for the initial 10-mm GL of 40 days (vertical bars), 30 days (upper thin line), and 60 days (lower thin line).



interval of GL (Table 3, Fig. 7). Assuming a daily deposition of growth increments, the curve was adjusted to the initial ages of 30, 40, and 60 days for the first 10 mm GL (not observed directly on the gladius surface) according to previous statolith age determinations for *I. illecebrosus* (Hurley and Beck 1979; Morris and Aldrich 1985; Balch et al. 1988). Gladius growth was found to be nearly linear after approximately 120 days (Fig. 7). Squid at this age attained 40–80 mm GL, which approximates the sizes they recruit to the trawl fishery on the continental shelf off Nova Scotia and Newfoundland (Rowell et al. 1985). We consider this size range to be the boundary between juvenile offshore and adult inshore phases, although sexual maturation initiates at larger sizes (around 200 mm ML; O'Dor 1983).

Reconstruction of gladius growth histories

Individual growth histories of the three captive squids are shown in Fig. 8 in relation to the temperature fluctuations and feeding events during the monitored period. The male RYO had its stomach full at the time of tagging and had a 26-g meal the next day. The animal was not seen feeding for the next 6 days and had, after this period, five consecutive meals (ranging from 4.5 to 24 g) in the following 5 days. RYO lived for another 11 days on an uncontrolled feeding schedule. The gladius growth series shows peaks in growth increment succeeding both feeding episodes observed (Fig. 8A). The male RGR did not eat until 9 days after being placed in the tank. In the following 3 days it had two meals (8.5 and 16 g) and fed freely for another 7 days until it died. Growth increments increased sharply after meals and remained high for another 4 days during the uncontrolled feeding period (Fig. 8B). The female ROR fed for the first time 8 days after capture. Five meals (ranging from 8 to 68 g) were taken in the following 8 days. ROR lived for another 43 days on an uncontrolled feeding schedule and was found to be in an advanced maturity stage at death. An increase in growth rate followed the feeding periods (Fig. 8C), especially after the second meal, which was the largest observed during the entire experiment (R.K. O'Dor, personal observation). This animal experienced a water

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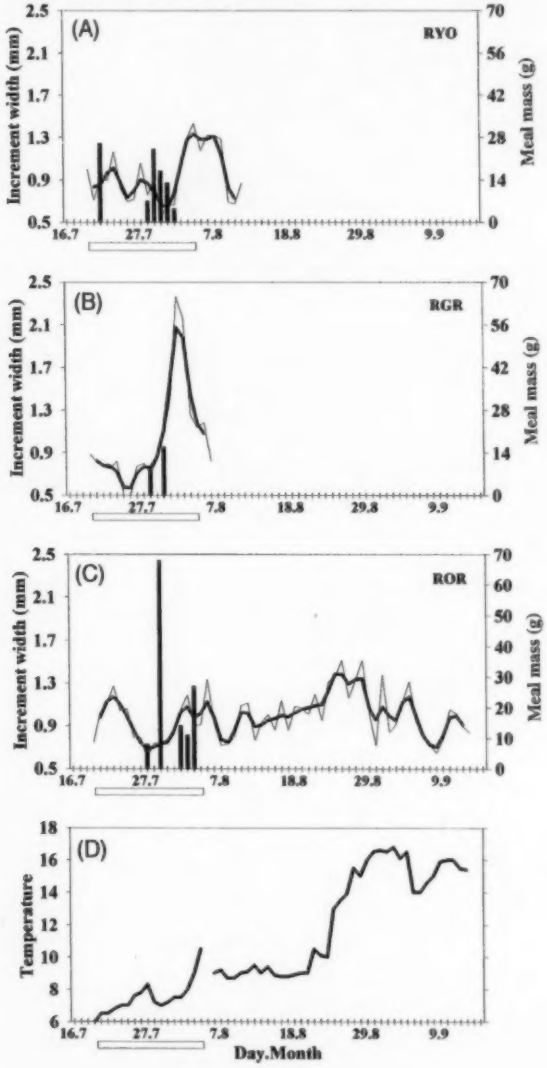
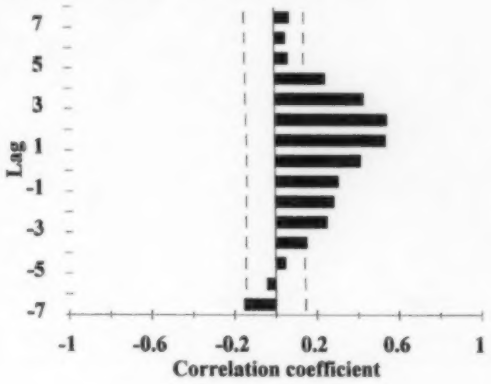
Fig. 8. Reconstructed individual gladius growth histories (increment width in millimetres) of three captive squids. (A) Squid RYO, male, 170 mm ML; (B) squid RGR, male, 195 mm ML; (C) squid ROR, female, 235 mm ML; (D) temperature (°C) fluctuation at the bottom of the tank. Increment width series (thin lines) were smoothed (thick lines) after the application of a statistical filter. Solid vertical bars represent feeding rates (in grams) in the tank during the controlled period from 19 July to 4 August 1978 (horizontal bars).



temperature increase after the feeding experiments (Fig. 8D). The temperature series cross-correlated with the growth increment series indicated a significant positive response of growth lagging the temperature variation by 2 or 3 days (Fig. 9). A general decrease in growth rate during the last 3 weeks, despite sustained high temperatures, was probably related to the fact that the animal was reaching sexual maturity and investing more energy into growth of gametes (Figs. 8C and 8D).

Fig. 9. Pattern of estimated cross correlation between the water temperature series moved over the gladius growth series of the female ROR. The broken lines represent the standard error. Higher positive correlations occur at 2- and 3-day lags.



Discussion

The age of an organism can be interpreted from the accretive growth of a skeletal structure provided that (*i*) the periodicity of increment deposition is known and (*ii*) the structure's time record includes all of the animal's lifetime. Furthermore, if the deposition rate of skeletal material is proportional to somatic growth, growth increments on the structure can be used to back calculate somatic growth on previous occasions (Campana and Neilson 1985). There are three previous evaluations of the gladius structure for age determination and growth studies in the ommastrephid squids *I. argentinus* and *Sthenoteuthis oualaniensis* and the loliginid *Sepioteuthis lessoniana* (Bizikov 1991; Arkhipkin and Bizikov 1991; Jackson et al. 1993). All of them concluded that (*i*) the gladius ostracum is likely unsuitable for age determination, (*ii*) the gladius increments are linearly related to mantle growth and may provide accurate estimates of individual size-specific growth rates, and (*iii*) at least during the adult stage, the deposition of gladius increments approximates daily periodicity. These same conclusions and further distinctions were evident in the present results on the gladius structure of *I. illecebrosus*.

The early growth of the gladius ostracum is progressively masked by structural modification as growth layers are continuously added to the ventral surface. Consequently, only a fraction of the growth increments can be enumerated in older animals, and the total age cannot be estimated. This posterior faint zone may already be present in the paralarval gladius, as the total number of visible increments in the single paralarval gladius examined was found to be nearly half the number of statolith daily rings counted in paralarvae of similar sizes (Balch et al. 1988). Further investigation, however, is needed because 14 days is not an unrealistic age for a 3.8-mm GL squid.

The gladius ostracum increment deposition was proportional to the lengthening of the mantle throughout juvenile and adult life. The relationship, however, was altered by the shrinkage of the mantle in preserved specimens. The gladius lies free in the shell sac along the dorsal midline of the mantle and is highly flexible. As the mantle shrinks the gladius tends to bow,

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Table 4. Daily size-specific growth rates (mm/day) reported in the literature, and calculated by statolith size-at-age analysis (s) and modal displacement of ML frequency distributions (m).

Species	ML range (mm)	Growth rate (mm/day)	Source
I. illecebrosus	10–190	0.38–1.43	This study
	30–110	1.26–1.43	Arkhipkin 1990 (s)
	110–235	1.33–1.43	
	117–245	0.88[a]	Dawe and Beck 1992 (s)
	116–215	0.66[b]	
	33–266	1.78	Morris and Aldrich 1985 (s)
	>140	0.33–1.33[a]	Squires 1967 (m)
		0.66–1.00[b]	
	8–120	0.66[a]	Mesnil 1976 (m)
	8–115	0.58[b]	
	143–220	0.67[a]	
	141–211	0.60[b]	
I. argentinus	<50	0.36–0.93	Arkhipkin 1990; Arkhipkin and Scherbich 1991; Arkhipkin and Laptikovsky 1994 (s)
	50–100	0.73–1.67	
	100–200	0.83–1.73	

[a]Data are for females only.
[b]Data are for males only.

the result being that it is longer than the projected ML (usually measured in most cephalopod studies). Although this effect can be reduced by using preserved–fresh ML correction factors, it is best to avoid mixing individuals preserved or handled differently when gladius increments are to be transformed into mantle growth rates. In general, because gladius growth correlates strongly with somatic growth and its length is less variable than ML, GL may be the best linear measurement of size (Kristensen 1983), and gladius growth increments are excellent indicators of size-specific somatic growth (Bizikov 1991; Arkhipkin and Bizikov 1991; Jackson et al. 1993; Bizikov 1995).

The enumeration of gladius growth increments was consistent in this study, and at least during the adult phase, growth increments seem to approximate daily mantle growth rates estimated independently. Mean growth increment widths along the gladius were similar to values obtained for *I. argentinus* (0.9–2.0 mm, GL range between 150 and 300 mm) and for *Sthenoteuthis oualaniensis* (0.77, 0.79, 1.25 mm, averages between 30 and 270 mm GL for males and medium-sized and large females, respectively; Bizikov 1991, 1995). Furthermore, *I. illecebrosus* gladius increments fell within the ranges of mean daily size-specific growth rates estimated from statolith analysis and ML frequency distributions (Table 4). Mean gladius growth rates inferred here were lower than statolith-determined rates for Scotian Shelf squid (Morris and Aldrich 1985; Arkhipkin 1990) and higher than rates obtained for squid from inshore Newfoundland (Dawe and Beck 1992), although minimum and maximum gladius increments overlapped those levels. Similar results were obtained in comparison with estimates for *I. argentinus* (Table 4). Mean growth increment widths estimated for gladii under 100 mm were less consistent and seem to overestimate increment widths directly measured (Perez and O'Dor 1994). This indicates that the estimation of gladius size-specific growth rates by Bizikov's (1991) relative growth method may not be accurate for juvenile squid gladii.

The growth reconstructed from gladius analysis covered nearly two thirds of the life of *I. illecebrosus*. The paralarval phase (GL <10 mm) and mature stages (GL between 200 and approximately 300 mm) were not included. A predominantly linear trajectory of GL was preceded by slower juvenile growth in accordance with curves reconstructed for other species from gladius growth (Bizikov 1991, 1995; Arkhipkin and Bizikov 1991). In the ommastrephid *S. oualaniensis*, when large mature squids were included, an S-shaped growth curve was obtained because of an additional phase of growth decline at the end of life (Bizikov 1995). The difficulties in fitting growth models to size at age plots largely derive from variability in individual growth (Jackson 1994). Because growth trajectories of individuals may vary considerably depending on environmental conditions, size at age plots of individuals sampled in different seasons or areas exhibit large variation. Size-specific growth rates of gladii measured on individual squid should provide more accurate descriptions for general growth models of squid.

At the individual level, the variation in growth increments in the gladius ostracum may be sensitive to feeding events, temperature fluctuations, and possibly sexual maturation. The complete digestion of a large meal of live fish by *I. illecebrosus* may require over 30 h at temperatures around 10°C (Wallace et al. 1981). Thus, growth responses lagging 3–5 days after feeding events, as suggested by the present results, do not seem unreasonable. Temperature directly affects metabolic and feeding rates (O'Dor and Wells 1987) and higher temperatures may cause a more immediate response in growth such as shown in the ROR female. Although the limited material available and the difficulty of reproducing such experiments with ommastrephid squids prevented further conclusions, gladius structure seems to have good potential as a tool for reconstructing individual growth histories and assessing internal and external factors affecting growth. The reconstruction of individual growth histories in adult and juvenile *I. illecebrosus* has revealed different growth performances of schools, sex groups, and geographical and seasonal subpopulations as well as small-scale horizontal growth rate variation (Perez 1994).

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Lunar cycles and the maturation process were additional factors detected in the variability of growth rates of female *S. oualaniensis* in the Arabian Sea (Bizikov 1995).

Finally, our results indicate that, during adult life, gladius increments and statolith rings are deposited with similar periodicity. In a similar analysis for *Sepioteuthis lessoniana*, Jackson et al. (1993) concluded that such proportionality was restricted to adult life. For *I. argentinus* and *S. oualaniensis*, daily periodicity was suggested on the basis of the similarity between growth trajectories estimated by both gladius and statolith analysis (Arkhipkin and Bizikov 1991; Bizikov 1991). Our direct comparison with statolith-determined age included a relatively small number of individuals of a limited size range, and showed considerable scatter in the data. Therefore, conclusions about the daily deposition of gladius increments should be considered with caution. Combined with the evidence provided by size-specific growth and the individual growth histories reconstructed for captive squid, however, the daily nature of gladius increments is supported. Additional direct validation (Natsukari et al. 1991) would still be useful.

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Efficiency of feeding on zebra mussel (*Dreissena polymorpha*) by common bream (*Abramis brama*), white bream (*Blicca bjoerkna*), and roach (*Rutilus rutilus*): the effects of morphology and behavior

Leo A.J. Nagelkerke and Ferdinand A. Sibbing

Abstract: We compared efficiencies of feeding on zebra mussel (*Dreissena polymorpha*) by three syntopic cyprinids: common bream (*Abramis brama*), white bream (*Blicca bjoerkna*), and roach (*Rutilus rutilus*). Empirical feeding efficiency (the ratio of food energy content to food capture and processing costs) is largely determined by the time needed to handle prey, rather than by the energy required for specific feeding actions, such as detaching and crushing the mussel. The highest individual feeding efficiency values were achieved by large white bream and roach, while common bream performed poorly. Maximal and optimal prey sizes were highest in roach. X-ray movies showed that in roach the dimensions of the oral gape, pharyngeal slit, and chewing cavity all matched, and thus limited, maximal prey size. In common bream and white bream prey size is limited by pharyngeal crushing power. The ability of roach to process larger mussels is based on the larger static crushing forces that can be exerted by its stout pharyngeal jaws; this could contribute to a strong position in competing for mollusks, most probably at the expense of its filter-feeding capacity. An integration of morphology, behavior, and performance helps to explain feeding efficiency differences among fish species and could eventually provide insights into mechanisms of trophic segregation.

Résumé : Nous avons comparé l'efficacité de la consommation de moules zébrées (*Dreissena polymorpha*) par trois cyprinidés syntopiques : la brème commune (*Abramis brama*), la brème bordelière (*Blicca bjoerkna*) et le gardon (*Rutilus rutilus*). L'efficacité alimentaire empirique (rapport de la teneur en énergie alimentaire aux coûts de capture et de transformation de la nourriture) est déterminée en grande partie par le temps nécessaire pour manipuler la proie plutôt que par l'énergie nécessaire pour des actions spécifiques de consommation, comme le fait de détacher et de broyer la moule. C'est chez les brèmes bordelières et les gardons de grande taille qu'on relevait les valeurs individuelles de l'efficacité alimentaire les plus élevées, tandis que la performance de la brème commune était minimal. La taille maximale et optimale des proies est la plus élevée chez le gardon. La cinéradiographie montre que, chez le gardon, l'ouverture de la bouche, la fente pharyngienne et la cavité masticatoire sont de dimensions correspondantes, et donc limitent la taille des proies. Chez la brème commune et la brème bordelière, la taille des proies est limitée par la puissance de broyage des máchoires pharyngiennes. La capacité du gardon à traiter des moules plus grosses tient aux forces statiques de broyage plus grandes que peuvent exercer ses solides mâchoires pharyngiennes; elle peut lui donner une position forte dans la compétition pour la consommation de mollusques, probablement aux dépens de sa capacité de filtrage. Une intégration de la morphologie, du comportement et de la performance aide à comprendre les différences dans l'efficacité alimentaire entre les espèces de poissons et pourrait même jeter une certaine lumière sur les mécanismes de la ségrégation trophique.
[Traduit par la Rédaction]

Introduction

Eutrophication in shallow Dutch lakes has considerably changed the environmental conditions of these aquatic ecosystems by creating turbidity and causing the disappearance of macrophytes (Hosper and Jagtman 1990). The population of common bream (*Abramis brama* L.) has increased considerably, while the number of two other closely related cyprinids has remained fairly constant (white bream, *Blicca bjoerkna* L.) or has decreased (roach, *Rutilus rutilus* L.) (Lammens et al. 1992). One of the proposed causes of these changes in species abundance is the shift in food composition associated with eutrophication. The increased production of phytoplankton and its primary consumers, zooplankton, as well as the subsequent effects on the fish fauna have been a subject of comparative research on trophic shifts and diet overlap between these three fish species (Lammens et al. 1987, 1992; Hoogenboezem et al. 1990, 1991; van den Berg et al. 1992). Apart from competing for zooplankton at standard lengths smaller than 15 cm, the three species also compete for chironomids. Food scarcity triggers the switch to feeding on zebra mussel,

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Dreissena polymorpha Pallas, by white bream and roach (Lammens et al. 1987). This study (*i*) investigates differences in the feeding efficiency on zebra mussels among common bream, white bream, and roach and (*ii*) analyzes the structural, functional, and behavioral causes of these differences to help explain the competitive position of these fish for food resources. Morphology (i.e., size and position of feeding structures affecting feeding efficiency on zebra mussels, such as oral and pharyngeal jaws) will be tested for its predictive value for the efficiency of molluscivory.

Feeding efficiency is defined as the ratio of energy gain from the mussel to the costs of capturing and processing it. Handling times have previously been used to estimate the energetic costs of feeding in fish (Werner 1974; Werner and Hall 1974; Slootweg 1987; Paszkowski et al. 1989). Drost and Van den Boogaart (1986) estimated the actual energy spent by larval carp in striking their prey and showed that it represented only a fraction of the energy gained. We will estimate how total handling time and its associated standard metabolic energetic costs for feeding on zebra mussels compare with the energy spent on the most demanding actions in the food processing sequence.

How does one explain differences in feeding efficiency? As in design and application of tools, morphological and behavioral specializations for the processing of a particular food type are supposed to increase energetic efficiency (and decrease overall handling time). Such specializations usually limit the effective utilization of other food types, since structural and functional demands for the efficient utilization of one type of food are mostly incompatible with that of a different type (Sibbing 1988, 1991*a*; Barel 1993; Wainwright and Richard 1995). Functional morphology provides the framework for such studies. Therefore, to explain differences in the efficiency of processing zebra mussels among common bream, white bream, and roach, the structure and functioning of their feeding system will be compared focusing on oral gape size, the dimensions of the pharyngeal slit and the gape of the chewing cavity, and the pharyngeal jaw parameters that play a key role in the mechanical breakdown of zebra mussels (Sibbing 1991*b*). Although oral gape size is generally taken as the major factor determining optimal prey size (Werner 1974), each of the former parameters may limit the size and toughness of mollusc shells that can be processed and thereby affect feeding efficiency. Dynamic parameters in feeding behavior were investigated using electromyography and X-ray movies while fish fed on mussels.

In general, the environmental food resources that can be actually utilized by fish are limited by the specific feeding structures and their functioning, and feeding behavior. From these parameters, potential food resources may even be predicted (Sibbing 1988, 1991*a*, 1991*b*; Norton et al. 1995; Wainwright and Richard 1995). These hypothesized potential food niches can be tested by comparing the available and the ingested food resources. Such an approach is applied in analyzing trophic segregation and reconstructing potential food webs for fisheries research (Sibbing et al. 1994). Availability of resources, competition, and predation will further determine fish species composition and abundances. The latter parameters are beyond the scope of the present analyses.

Materials and methods

Fishes and zebra mussels

Three common bream (*Abramis brama* L., 155–298 mm standard length (SL)), four white bream (*Blicca bjoerkna* L., 128–246 mm SL), and three roach (*Rutilus rutilus*, 146–235 mm SL) were collected in Lake Tjeuke (The Netherlands) at least 1 month prior to the experiments. Two weeks before starting the experiments, fish were kept separately in aquaria of approximately 50 × 40 × 40 cm at 20°C. In this period fish were fed only zebra mussels (ad libitum).

Zebra mussels (*Dreissena polymorpha* Pallas) were collected monthly in Lake IJssel (The Netherlands) and kept at the laboratory in running, copper-free tap water of 10°C. Maximal length (L) of the total mussel and maximal width (W) of the two valves, perpendicular to the hinge between them, were measured to the nearest 0.05 mm, using vernier calipers.

X-ray cinematography and video recording

To analyze the intra-oral events during food processing, lateral and dorsoventral X-ray pictures of feeding fish were taken according to a modified version of Sibbing's (1982) method. Fish were trained to feed in a 2.5-mm perspex cuvette (100 × 9 × 25 cm) to reduce the X-ray absorbing water layer around the fish (cf. Hoogenboezem et al. 1990). The water level was 15 cm for the lateral and 10 cm for the dorsoventral X-ray images. Occasionally prey were injected with $BaSO_4$ paste to improve contrast in the images. A Philips Super 100 V X-ray apparatus (60 kV, 120 mA, 3 ms) produced the images, which were electronically intensified and recorded by an Arriflex 35-mm movie camera at 25 frames/s, using Scopix RP1 film (for recording periods up to 2 min). For longer sequences (up to 10 min) X-ray video recording at 50 kV and 1000 mA was used. The distance between X-ray tube and image intensifier was 85 cm. Regular video recordings were simultaneously made for external observations using standard equipment.

Electromyography

To record handling times and to differentiate among the consecutive feeding actions in the three species, electromyography (EMG, Fig. 2) was performed, extrapolating the results of Sibbing (1982) and Sibbing et al. (1986) for common carp. EMG also allows the duration and frequency of specific feeding actions to be measured. Briefly, after the fish was anaesthetized in a 100 mg/L solution of MS-222 (Sandoz, Basel) in water, 50-μm insulated copper wire electrodes were inserted in the key muscles, (*i*) *musculus levator operculi* (LOP) and (*ii*) *musculus levator arcus branchialis V* (LAB V), indicating mouth opening and chewing activity, respectively, and in the (*iii*) palatal (PAL in Fig. 1) and (*iv*) postlingual organs, active in internal manipulation and transport. The amplification factors used were 5000 or 10 000. The low and high cutoff frequencies were 100 and 5000 Hz, respectively. Signals were recorded by a Bell and Howell tape recorder (type CPR 4010) and an ink-jet recorder (Siemens; type S).

Video movies of the foraging fish were made synchronously with EMG to match external observations with internal events as apparent from muscle activities.

Estimation of feeding efficiency

The absolute feeding efficiency (AFE) was defined as

$$(1) \quad \text{AFE} = \frac{\text{total energy benefits from food}}{\text{total energy costs of feeding}}$$

Energy benefits from food

The total energy content of a mussel (E_{mu}, in joules) was calculated using the equation of Prejs et al. (1990):

$$(2) \quad E_{mu} = 0.014\, L^{2.52}\,(3.74 \ln(L) + 7.65)$$

in which L is the length of the mussel (in millimetres).

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The fish cannot utilize 100% of the energy content of the mussel. Phillips (1969) and Brett and Groves (1979) estimated that between 69 and 74% of the energy contents of dietary protein can be utilized. E_m, the energy benefits from a mussel, is therefore conservatively estimated as

$$E_m = 0.5\, E_{mt} \quad (3)$$

Energy costs of feeding

A generalized feeding sequence in cyprinids includes search, detection, pursuit, intake, taste selection, size selection, transport, mastication, deglutition (the actual moment of swallowing, when food passes from pharynx into oesophagus), and digestion (Sibbing et al. 1986; Sibbing 1991*a*). The total energy spent on feeding (E_{fi}) can be written as

$$E_{fi} = (E_{search} + E_{detection} + E_{pursuit}) + E_{intake} + E_{selection} + E_{transport} + E_{mastication} + E_{deglutition} + (E_{digestion}) \quad (4)$$

The energy components in parentheses are not taken into account in this study. In the case of feeding on zebra mussels, the time and energy spent on search and detection are unknown and likely to be highly variable (even if hunger conditions are comparable), owing to a variable density and distribution of the mussels in the environment. Pursuit is not required. Therefore, the feeding process is studied from the point the fish detaches the mussel from its substratum to deglutition.

The time needed to handle a prey is generally accepted as a good parameter for estimating the energetic requirements during feeding (Werner 1974; Werner and Hall 1979; Slootweg 1987; Paszkowski et al. 1989). The minimum amount of energy required by the fish during feeding can be calculated by multiplying the handling time (T) by the standard metabolism (R_s, in watts) for cyprinid fishes at 20°C, as determined by Winberg (1956):

$$R_s = 0.47\, M^{0.8 \pm 0.044} \quad (5)$$

in which M is the body mass of the fish (in kilograms).

During feeding, the energetic requirement of a fish will be greater than R_s, but less than the maximal metabolic rate, which can be as high as 10–15 times R_s (Peters 1983). It is not known by what energy factor (between 1 and 15) R_s has to be multiplied to account for the total energetic costs of feeding. When comparing the energetic costs of feeding, the energy factor is assumed to be similar for our three species and even if it differs it is of less influence than the handling times, as will be shown later.

When considering the relative importance of extra energetic costs (on top of the metabolic energetic requirements) for the subsequent feeding actions, the energy to pull the mussel from its substratum, i.e., to break all its byssal threads (E_p, part of E_{intake} in eq. 4), and the energy to crush its shell (E_{cr}, part of $E_{mastication}$ in eq. 4) are most important, because they are the most strenuous. $E_{selection}$ will be small, as mussels are unsoiled and large, therefore internal selection is not required. $E_{transport}$ will also be small, since mussels are not struggling prey. We estimated E_p and E_{cr} using an equation for the breaking energy (E_{br}, in joules) of an elastic material (cf. byssal threads and shell), derived from Gordon (1976):

$$E_{br} = 0.5\, F_{br}\, s_{br} \quad (6)$$

in which F_{br} is the breaking force (in newtons) and s_{br} is the deformation of the material at the breaking point (in metres). Since the fish does not have a muscle efficiency of 100%, but rather its efficiency is estimated at 20% (Alexander 1977), the energetic cost (in joules) of pulling and crushing for the animal has to be corrected:

$$E_{p,cr} = \frac{0.5}{0.2} F_{p,cr}\, s_{p,cr} = 2.5\, F_{p,cr}\, s_{p,cr} \quad (7)$$

The extra amount of energy required for detaching and crushing, calculated by this formula, will be shown to be so small compared with the standard metabolic energetic requirements during the feeding process from intake until deglutition (E_s) that it can be neglected in E_{fi}. Empirical feeding efficiency (FE) is then calculated as

$$FE = \frac{E_m}{E_s} = \frac{E_m}{R_s\, T} = \frac{E_m}{0.47\, M^{0.8}\, T} \quad (8)$$

in which T (in seconds) is measured from the moment of intake to the moment of deglutition.

Handling time experiments

From observations of fish detaching mussels from stones, the adherence strength appeared to be variable and not directly correlated with the size of the mussel (in contrast with Prejs et al. 1990). For this reason and because of practical problems in presenting mussels attached to substrate, detached mussels were measured and presented one by one to the fishes. Handling times were measured (to the nearest second) from the moment of intake of the prey until the moment of deglutition, following mastication.

After verifying that the internal food handling actions can be accurately matched with external observations of the fish by means of X-ray video and electromyography, handling times were measured by observing the feeding fishes using a stopwatch or by analysis of the EMG recordings. Pauses in handling were subtracted if they did not exceed 30 s; apparently interrupted handling sequences showing longer pauses were discarded. Handling times were measured after the fish were starved for 24 h. No more than five prey items were presented on a single day of experiments, because a decrease in motivation and long pauses could be observed if more prey items were offered, probably because of satiation (cf. Werner 1974).

Measuring crushing energy

To determine the energy required for breaking *D. polymorpha*, whole mussels were placed on a Sartorius MP1405 electronic balance and crushed by means of a brass stamp with a flat surface that could be accurately manipulated vertically. The mussels were crushed in the same direction as they were in the chewing cavity (Fig. 1) of the fishes as determined from the X-ray recordings (Fig. 3), perpendicular to the plane between left and right valves. The accuracy of the force measurements was approximately 0.5 N (50 g on the balance). The deformation needed to break the shell (s_{cr}) was measured to the nearest 0.02 mm, using electronic vernier calipers (Sylvac SA), connected to the brass stamp. These measurements were corrected for the amplitude of the balance itself.

Measuring structural parameters associated with feeding

To compare structural specializations and limitations in the food handling of the three fish species, the maximal dimensions of their horizontal and vertical oral gape (G_h and G_v, respectively), the most limiting dimension of their pharyngeal slit (i.e., the vertical dimension, PS), as well as pharyngeal jaw and teeth parameters were studied (PJ and PT in Fig. 1).

G_h, G_v, and PS were not measured directly in the experimental fishes but were calculated from linear equations, derived from measurements on a size series of each of the three species. G_h and G_v were measured with vernier calipers on fresh, dead fish. To measure PS, their heads were fixed in a maximally expanded condition; after the gill arches were removed, the gill covers were sewn to the head and fluid dentist's rubber (Xantopren) was injected into the mouth. After coagulation of the rubber and dissection of the heads, casts of the oral, buccal, and pharyngeal cavities were acquired. PS was measured from these casts with vernier calipers. The maximal dimension of the chewing cavity (CC in Fig. 1), between pharyngeal teeth (PT) and chewing pad (CP), could not be measured directly, but it could be derived from X-ray recordings when fishes were trying to process a prey equal to, or larger than, the maximal size of their chewing cavity (Fig. 3).

The dry mass of the pharyngeal jaws was weighed. This value is considered as a comparative measure of the forces that can be transmitted to the food and reabsorbed by the jaws. The impact angles of anterior and posterior pharyngeal teeth on the chewing pad were measured as well. This is the angle between the chewing pad surface

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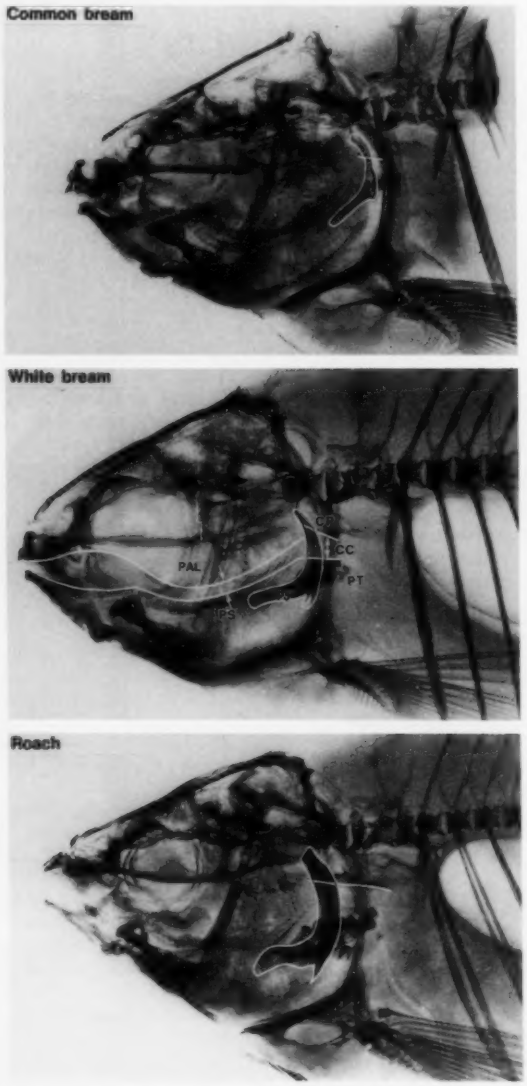
Fig. 1. X-ray pictures of the heads of common bream, white bream, and roach showing feeding structures and measuring parameters. Pharyngeal jaws (PJ) and the chewing pad (CP), fixed in the base of the skull, are marked by white outlines in all three species. In the picture of white bream, outlines of the mouth and pharyngeal cavities are drawn, as well as the palatal organ (PAL), the muscular roof of the pharyngeal cavity, the pharyngeal slit (PS, indicated by arrows), the chewing cavity (CC, indicated by arrows), and the pharyngeal teeth (PT). Note that common bream's pharyngeal jaws and teeth are the smallest and absorb X-rays the least, while those of roach are very large and dense. Those of white bream are intermediate.



and the length axis of the occluding teeth. The angle changes from the anterior to the posterior teeth in relation to different masticatory operations (Sibbing 1991*b*). Crushing requires forces perpendicular to the chewing pad and bending moments on the teeth are best avoided by aligning their axes parallel to these forces. Thus, large impact angles of teeth reflect their appropriateness for crushing.

Standard length (SL), head length (HL), and body mass of the fish (*M*) were used as reference parameters. These parameters and the other symbols used in this paper are listed in the Appendix.

Statistical analysis

Differences among the three species were analyzed using analysis of variance (ANOVA) for handling times, the regression coefficients of the handling time equations, the feeding efficiencies, and the durations of the mastication strokes. Kruskall–Wallis's nonparametric test was used for the analysis of differences in structural parameters, muscle activity frequencies, mastication intensity, and the optimal and maximal prey widths. For both methods the accepted level of significance was $p < 0.05$.

Results

Consecutive actions in feeding on zebra mussels

In trial experiments zebra mussels that had attached to stones were presented to all three species to observe their detaching behavior. All fishes tried to take in a mussel by a sequence of very rapid suction movements. If within 2 s the mussel was not detached from its substratum, the fish would neglect it and try another mussel. Grasping a mussel and tearing it from the substratum was never observed. Detachment time hardly influenced the total handling time (up to 120 s) and for the purpose of standard comparison in further experiments detached mussels were measured and presented one by one.

The (particulate) intake of a detached mussel involves rapid suction movements (cf. Sibbing 1991*a*) similar to those used for detaching the mussel. In the electromyogram (Fig. 2) this is indicated by high LOP activities, opening the mouth, synchronous chewing activity in the LAB V being absent. After intake, internal manipulation of the mussel was clearly observed in the X-ray pictures. Manipulation served (re)positioning the mussel and usually involved backwashing (cf. Fig. 3*b*) by closed protrusion (short, low-amplitude bursts of LOP activity, Fig. 1). Sometimes the mussel was spat out and immediately taken in again. After being repositioned, the mussel was transported in a caudal direction by peristalsis of the muscular palatal and postlingual organs (cf. Sibbing et al. 1986) and loaded into the expanded chewing cavity for crushing (Fig. 3*a*; start of LAB V activity in Fig. 2). In all successful intakes the mussel was eventually positioned with its length (*L*) in rostrocaudal orientation (Fig. 3*a*) and its width (*W*, its minimal dimension, perpendicular to plane between the valves), dorsoventrally oriented (Fig. 3*c*). Mastication of the prey was readily observed externally by the repetitive and strong upward rotation of the skull and synchronous opening of the mouth (cf. Fig. 3*a*). This was reflected by repetitive LOP activity and synchronized LAB V activity, the latter being the largest chewing muscle (Fig. 1). If a fish could not load a mussel completely into its chewing cavity, it spat it out and neglected it (cf. Fig. 3*c*).

Finally, deglutition of both flesh and the broken shell parts was observed in the EMG as a short and low LAB V activity (<0.1 s). The whole sequence of feeding actions resembled food processing in carp (Sibbing 1982, 1988; Sibbing et al. 1986) and was similar in all three species, differing only in the duration and frequency of muscle activities.

By combining X-ray movies, video recordings, EMGs, and

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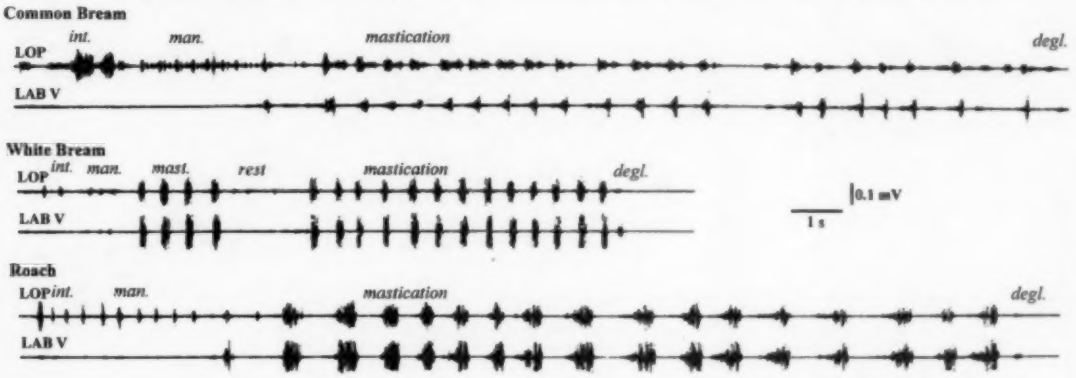
Fig. 2. Electromyograms of feeding on *Dreissena polymorpha* by common bream (SL 298 mm), white bream (SL 235 mm), and roach (SL 235 mm). LOP, *musculus levator operculi*; LAB V, *musculus levator branchialis* V; int., intake; man., manipulation; mast., mastication; degl., deglutition. Patterns of the three species are similar, but note the differences in frequency and duration of the masticating strokes.



external observations, we concluded that the complete sequence of internal feeding actions could be discriminated externally. Handling times for different feeding actions could be measured accurately from freely feeding fishes, without disturbance from experimental devices attached to the fish.

Quantitative differentiation within the feeding sequence

T can be divided into manipulation time (including rinsing, positioning, transport, and loading activities) and masticatory time (total sequence of masticatory cycles). The width of the mussel, ranging from 44 to 100% of the maximal prey width that was taken (W_{max}), does not significantly change the proportion of time required for manipulation and mastication. This proportion is also not significantly different among the three species (Fig. 4). With larger prey, mastication seemed to be interrupted more often than with smaller prey and repositioning occurred more often (data not shown).

The average frequency of mastication strokes (Fig. 4) was significantly lower in roach (1.17 Hz) than in common bream (1.89 Hz) and white bream (2.25 Hz). The difference between the latter two is also significant. The duration of the masticatory strokes (Fig. 4) was significantly larger in roach (0.266 s) than in common bream (0.091 s) and white bream (0.131 s) and the difference between the latter two species was also significant.

To estimate mastication intensity, the frequency and duration of mastication strokes were multiplied. The average intensity was significantly lower for common bream (0.170), while white bream and roach showed similar intensities (0.294 and 0.308, respectively, Fig. 4), although achieved in different ways.

Handling times and prey size

The observed handling times (Fig. 5) are described best by (significant) single-logarithmic functions of the mollusc width (W): $T = a \exp^{bW}$, in which a and b are constants (Table 1). Overall, T increased significantly with W and decreased significantly with fish standard length. T was smallest in white bream and largest in common bream. All of these differences of T among the three species were significant.

Constant b from the handling time function (Table 1) decreased significantly with increasing fish standard length. Overall, it was significantly larger for common bream than for white bream and roach, but there was no significant difference between white bream and roach. This means that (*i*) in small fish the increase of T with W was more rapid than in large fish, and (*ii*) in common bream the handling time increased more rapidly with W than in the other two species (cf. Fig. 5).

The maximal prey size that could be processed (W_{max}, Table 2) was significantly smaller in common bream (mean of 2.2% of SL) than in the other two fish species. W_{max} appeared to be larger for roach (mean of 4.1% of SL) than for white bream (mean of 3.7% of SL), but this difference was not statistically significant.

Cost/benefit ratio

To estimate the cost/benefit ratio of eating zebra mussels it is necessary to calculate the actual energy benefits from the mussel (E_m) and compare them with the standard energetic requirements during the feeding process (E_s), and with the required extra pulling (E_p) and crushing (E_{cr}) energy.

E_m was calculated from eqs. 2 and 3 for a series of 241 mussels of which both L and W were measured. The relation of E_m and W was best described by a power function (Fig. 6). E_s was calculated by multiplying R_s (eq. 5) by T (Fig. 6).

To calculate E_p using eq. 7 it was assumed that the deformation of the byssal threads at the breaking point (s_p) was 10% of the total byssal thread length (Vincent 1982). The length of the byssal threads was taken as 10% of the mussel length L, which was probably too long, but in this way the required energy was not underestimated. Values for F_p (i.e., the force required to tear a mussel from its substrate, thereby breaking or detaching all its byssal threads) were calculated from an empirically determined equation from Prejs et al. (1990). With these assumptions E_p was calculated using a series of 241 mussels for which both L and W were measured. The relation of E_p and W was best described by a power function (Fig. 6). The values for E_{cr} were directly calculated from the measured crushing force (F_{cr}) and s_{cr} and were best described by a power function (Fig. 6).

E_s ranged from 4.1 to 29.3% of E_m for common bream, from 1.0 to 8.2% for white bream, and from 1.3 to 8.3% for roach.

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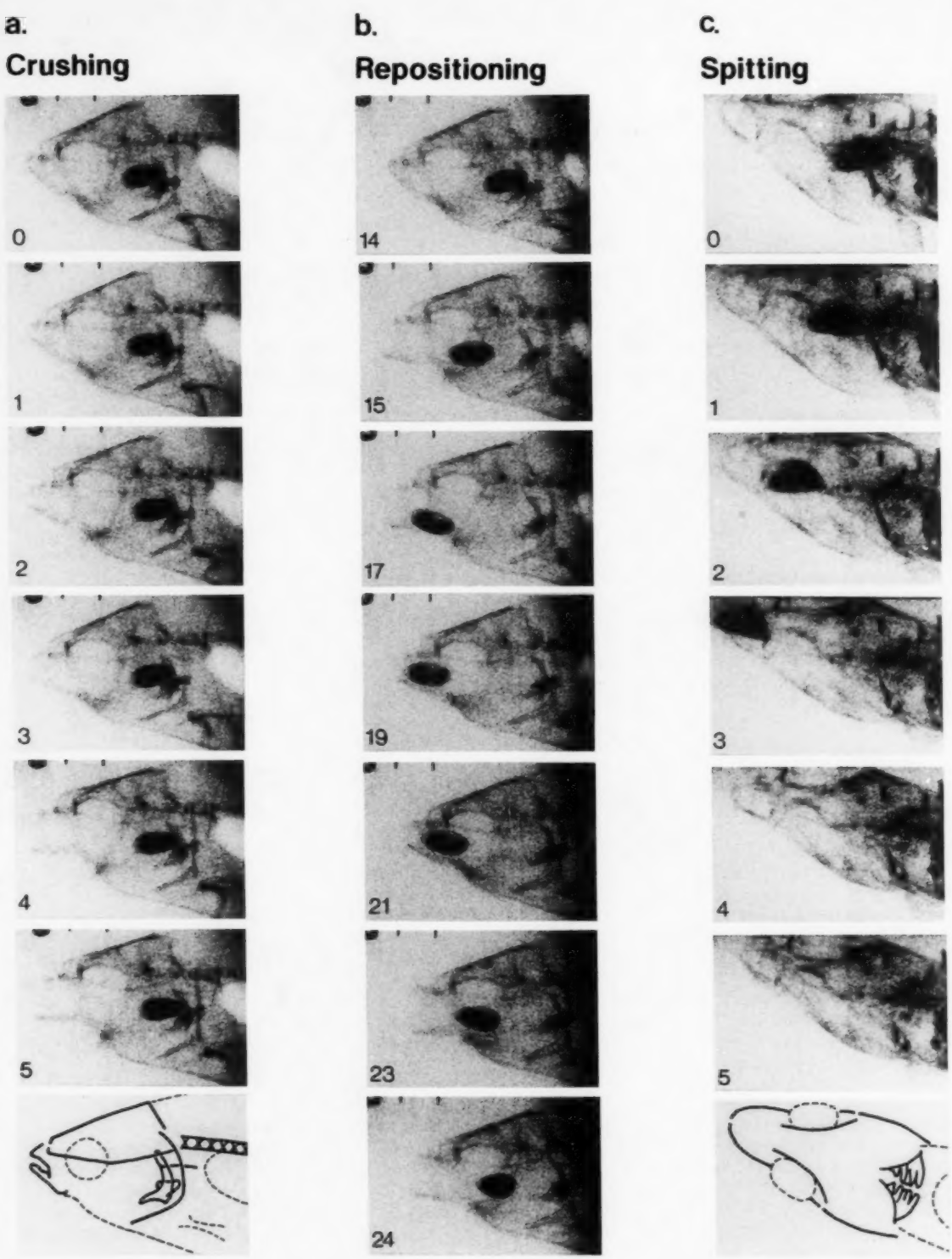

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Fig. 3. X-ray frames (25 frames/s) of the internal processing of a mussel (injected with $BaSO_4$) by roach (SL 207 mm). Line drawings indicate position of eyes, oral jaws, pharyngeal jaws, chewing pad, and gill covers. Numbers refer to the time sequence of the frames. (*a*) Lateral X-ray frames showing an attempt to crush a mussel, which fails because the prey could not be fully loaded into the chewing cavity. Note the lifting of the skull and opening of the mouth during the crushing stroke (frames 3–5). (*b*) Repositioning of the same mussel through backwashing to the oral cavity. (*c*) Dorsoventral X-ray frames of spitting out of a mussel after several attempts to load it into the chewing cavity. Note the adduction of the gill covers to reverse the water flow. These frames were used to determine the orientation of the mussel in the chewing cavity and the maximal size of the chewing cavity.

This shows that the energetic benefits from eating a mussel by far exceeded the energetic requirements for handling and processing it. The values of E_p and E_{cr} were even smaller than E_m (Fig. 6, note the logarithmic scale). E_p ranged from 0.059 to 0.15% of E_m and E_{cr} ranged from 0.0096 to 0.014%. E_p and E_{cr} were also much smaller than E_s (as is evident from Fig. 6): they ranged from 0.38 to 8.26% and from 0.04 to 0.95% of E_s, respectively. This justifies ignoring E_p and E_{cr} when calculating the cost/benefit ratio of eating zebra mussels.

Empirical feeding efficiency

FE was calculated from eq. 8 using E_m (Fig. 6), the mass (M) of the fish, and the handling time (T) for eating a mussel (Fig. 5). FE values are indicated by symbols in Fig. 7; the curves will be discussed later. FE increased significantly with fish size for common bream (Fig. 7, Table 1). Common bream showed the lowest FE values (maximal mean 17.37 for SL of 298 mm) and white bream the highest (maximal mean 55.13 for SL of 235 mm), although the difference between white bream and roach (maximal mean 36.65 for SL of 146 mm) was not significant.

The prey width at which the maximal FE was observed (empirically) was the optimal prey size (W_{opt}) (Table 1, Fig. 7). W_{opt} expressed as a percentage of SL was significantly smallest in common bream (mean of 1.4% of SL). There was no significant difference between white bream (mean of 2.9% of SL) and roach (mean of 3.0% of SL).

Theoretical feeding efficiency

FE was calculated by substituting the measured values of T in eq. 8. Instead of this we could also use the regression equations that express T as a function of W (Table 1), giving us the theoretical feeding efficiency (FE_*) (shown as curves in Fig. 7):

$$(9)\quad FE_* = \frac{1.131\,W^{2.322}}{0.47\,M^{0.8}\,a\,\exp^{(bW)}}$$

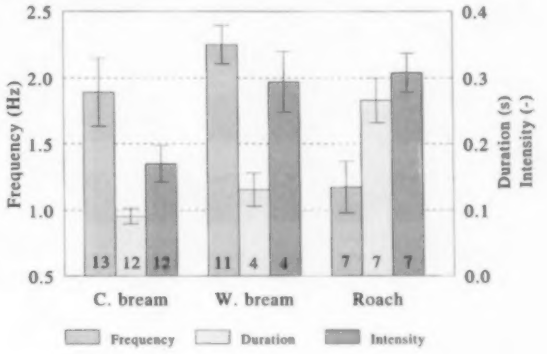
To determine the theoretical optimal prey size (W_{opt*}, in millimetres) the maximal value of eq. 9 has to be determined by first differentiating the function and then equalizing it to 0. This results in

$$(10)\quad W_{opt*} = \frac{2.322}{b}$$

W_{opt*} might be a better representation of optimal mussel width than W_{opt}, because the latter can be a single extreme value, while W_{opt*} is determined by the whole data set. W_{opt*} ranged from –33.0 to +29.5% from W_{opt}; the mean deviation was –3.14%.

W_{opt*} was smallest for common bream (mean of 1.2% of SL), intermediate for white bream (mean of 2.7% of SL), and largest for roach (mean of 3.2% of SL), mirroring the results for W_{opt}, although the difference between white bream and roach was larger for W_{opt*} than for W_{opt}.

Fig. 4. Electromyographic data of feeding on zebra mussels, ranging from 44 to 100% of the maximal prey size (W_{max}), by common bream (SL 298 mm), white bream (SL 246 mm), and roach (SL 235 mm). For each feeding sequence (eating one mussel) the frequency and mean duration of mastication strokes as well as the mastication intensity (duration times frequency) were calculated. The total means and standard deviations are shown, as well as the number of feeding sequences (bottom of bars). Note the low crushing frequency for roach and the high hammering frequency for white bream. The duration of mastication strokes is far larger in roach. This results in about equal mastication intensities in white bream and roach, while the intensity is far lower in common bream.



Morphological parameters limiting feeding

The horizontal and vertical oral gape (G_h and G_v, respectively) as well as the limiting vertical dimension of the pharyngeal slit (PS, Fig. 1) were described as linear functions of the head length (HL) (Fig. 8). G_v was always larger than G_h; thus, G_h may limit prey size. The length (L) of the mussel was not critical, since it was taken in along its length axis. W and H were approximately the same (W was 88–120% of H), so W can be compared with G_h.

G_h did not limit W_{max} in common bream (43–47% of G_h) nor in white bream (76–82%), but in roach (85–100%) G_h may well have limited prey size (Table 2). The same result was found for PS: W_{max} was 32–34% of PS for common bream, 65–83% for white bream, and 79–97% for roach.

The maximal dimensions of the chewing cavity, between chewing pad and pharyngeal teeth, could not be measured directly. From X-ray movies, however, it appeared that in roach a prey of size W_{max} could only just be loaded into the chewing cavity, with the pharyngeal jaws maximally spread and depressed (cf. Fig. 3). If after several trials a prey still could not

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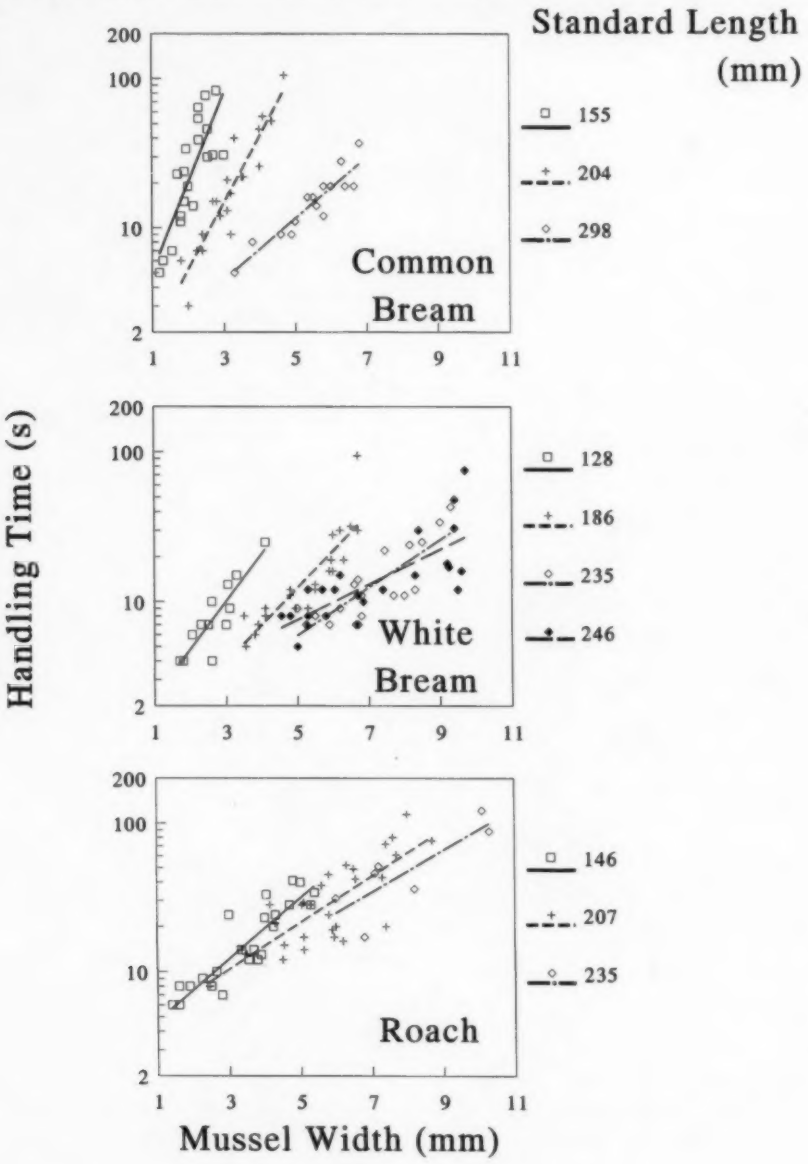
Fig. 5. Handling times (T) of all experimental fish versus mussel width (W, in millimetres). Note the logarithmic scale on the handling time axis. Coefficients of the regression equations are listed in Table 1. Note that for mussels of equal W the handling times for common bream are much longer than those of the other species and that for constant W, T is inversely related to fish size in all species.



be loaded successfully it was spat out (Fig. 3*c*). For such prey items W was slightly larger than W_{max}. Since W_{max} in roach was approximately of the same size as G_h and PS, all of the parameters considered limited prey size about equally, though the chewing cavity ultimately limited prey size. In common bream and white bream all mussels taken were easily loaded into the chewing cavity, suggesting that the size of the chewing cavity was not limiting prey size.

Common bream had the smallest mean pharyngeal jaw mass/body mass ratio (5.3×10^{-4}, Fig. 9). The mean ratio of white bream was about twice as large (10.6×10^{-4}), and for roach it was again about twice as large as that of white bream (19.0×10^{-4}) (cf. Fig. 2). Only the difference between common bream and roach was significant.

The impact angle of the most anterior crushing teeth was significantly larger in roach (average of 80°) than in common bream (average of 56°); the differences between white bream (average of 72°) and common bream and between white bream

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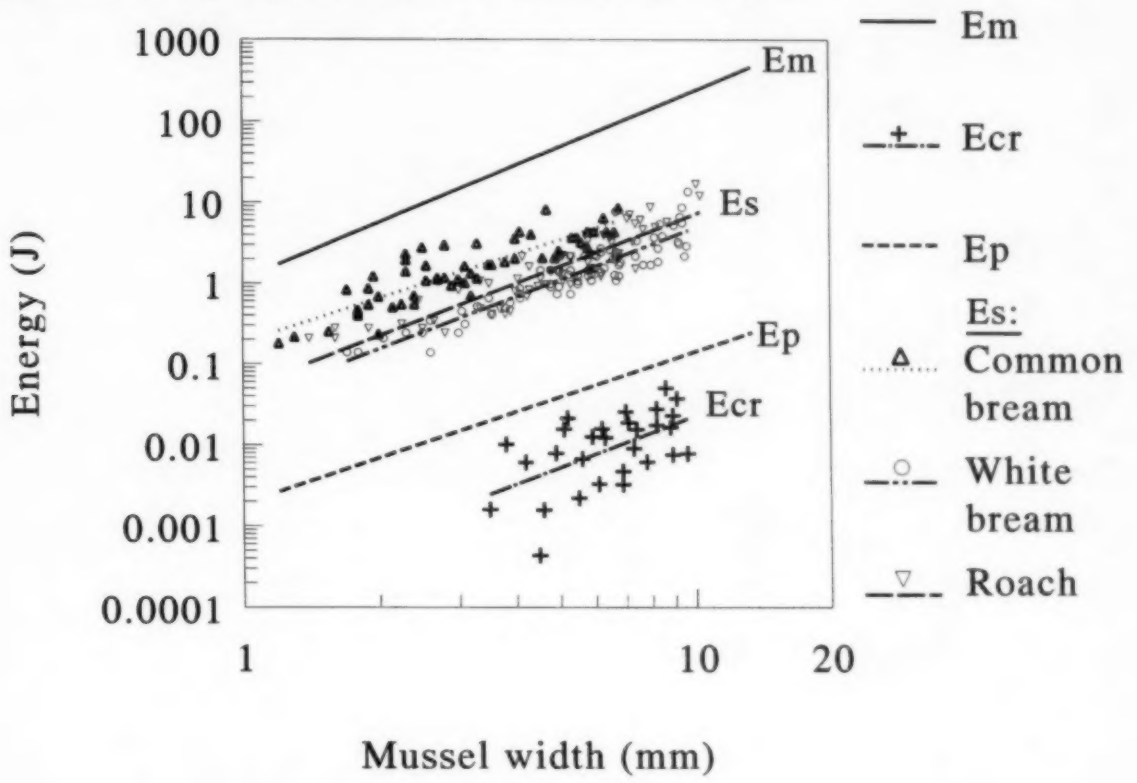
Fig. 6. The estimated energy content of mussels ($E_m = 1.131\ W^{2.322}$) and the energy required to pull the mussel from its substratum ($E_p = 0.001\ 89\ W^{1.876}$) calculated from equations from Prejs et al. (1990) for the relationships of mussel length (L) with total energy content (eq. 2 in the text) and pulling force ($F_p = \exp(0.127\ L - 3.437)$. The energy required to crush the mussel ($E_{cr} = 0.000\ 168\ W^{2.136}$, $R^2 = 0.32$, $n = 29$) and the standard metabolic energy requirements during handling time (E_s, pooled per species) complete the comparison. Note the double logarithmic scale and that E_p and E_{cr} are only a small fraction of E_m, while E_s is much more important.



and roach were not significant (Fig. 9). There were no significant differences in the impact angle of the most posterior teeth among the three species (Fig. 9). The large relative jaw mass and anterior impact angles both suggest that roach is more suited for exerting large crushing forces.

Discussion

Energetic efficiency of feeding

This study estimates the actual energetic costs and benefits of feeding. It shows that the time needed to handle a prey is energetically more costly than specific feeding actions. This validates the assumptions of previous authors (Werner 1974; Werner and Hall 1974; Mittelbach 1981) that T is a good estimate for comparing energy expenditure during feeding.

The importance of T is shown by comparing the standard energetic requirements during handling time (E_s) with the extra energetic costs of detaching the zebra mussel from its substratum (E_p) and crushing it (E_{cr}) (Fig. 6). E_s can consume a considerable proportion of the energy gained by feeding on zebra mussels (E_m) (1–29%). Taking into account the fact that the metabolic rate of an active (e.g., feeding) fish is higher than R_s, the metabolic energetic requirements during handling of a prey consume an even more important proportion of E_m. Detaching the mussel from its substratum and crushing it are probably the most demanding actions that are performed by molluscivorous cyprinid fish, but still they only cost a fraction of E_m (E_p ranges from 0.059 to 0.15% of E_m, and E_{cr} from 0.0096 to 0.014% of E_m). Therefore, it would be profitable for a fish to maximize prey ingestion per unit of time rather than minimizing the E_p/E_m or the E_{cr}/E_m ratio (as argued by Prejs et al. 1990).

Evidence that the energy content of a prey is much larger than the cost of specific feeding actions has been found previously (e.g., sucking in of prey by carp larvae: Drost and Van den Boogaart 1986). The large energy content of prey makes it possible for a fish to exploit prey of suboptimal size profitably, because even when E_m/E_s is not maximized, it can still be large enough to sustain the fish. Therefore, at low environmental densities of zebra mussels, a fish that can extend the range of utilizable prey beyond the range of others (e.g., by being able to eat larger mussels than others, because of its mechanics of prey handling and processing) may be at an advantage.

Handling time, feeding efficiency, and prey size

Handling time by a particular fish size increases with mussel

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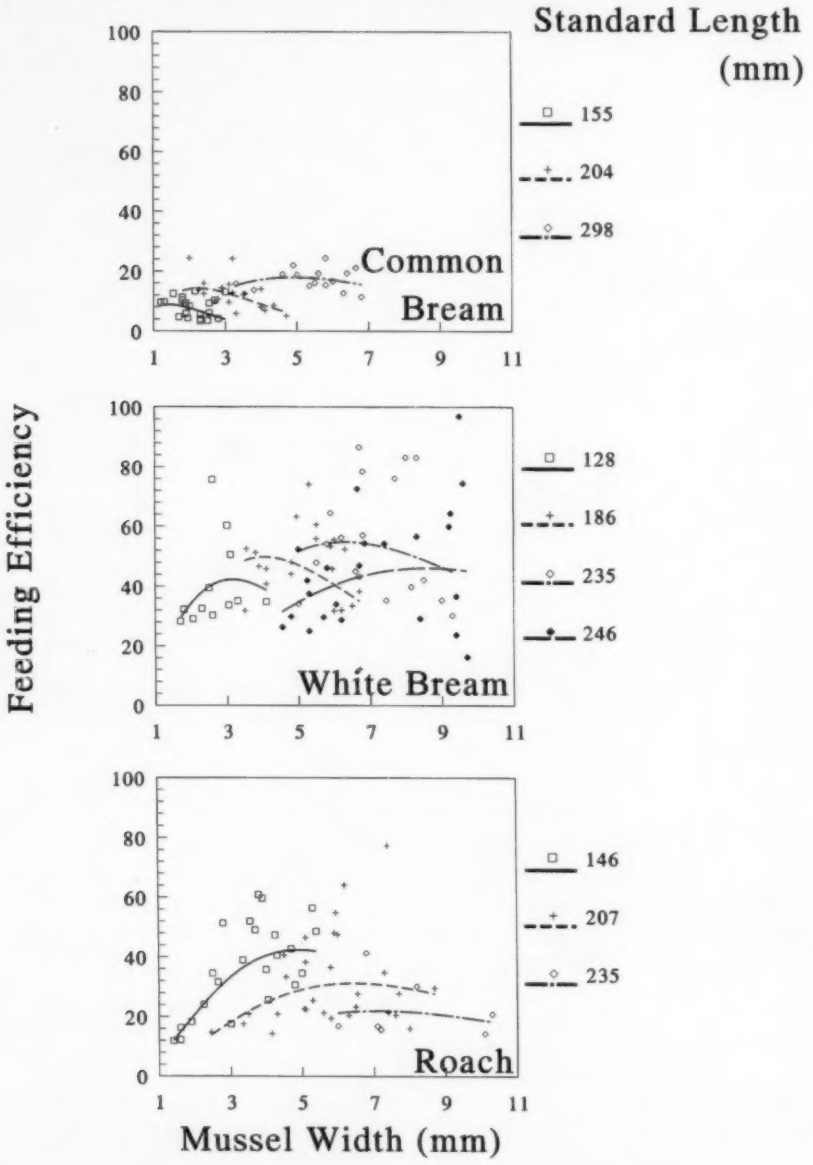
Fig. 7. Empirical feeding efficiency (FE) data of all experimental fish, calculated from the actually measured handling times (T, Fig. 5). The curves show the theoretical feeding efficiency ($FE_\bullet$) and are calculated using handling times derived from the regression data (Table 1, for further explanation see the text). Note that FE values for common bream are much lower than for the two other species.



size, decreases for a given mussel size with increasing fish size, and differs among fish species, increasing from white bream towards roach and common bream. Consequently, empirical feeding efficiency (FE) for a given mussel size increases with fish size (only significantly in common bream) and increases from common bream towards roach and white bream. Common bream is far less efficient in eating zebra mussel than white bream and roach (mean FE values of common bream range from 8 to 17, compared with 40–55 for white bream and 22–37 for roach; Table 1).

If we were to try to estimate the FE values that are high enough for profitable feeding on zebra mussels, we have to take into account that the actual energetic requirements during (active) feeding are higher than $R_s T$, and also that periods and actions when the fish is not feeding cost energy. The peak metabolic rate can be 10–15 times R_s (Peters 1983). Estimates of the average metabolic rate in the field vary. Adams et al. (1982) gave values of 1.18–2.44 times R_s for largemouth bass (*Micropterus salmoides*), Ware (1975) estimated a factor of 3 for bleak (*Alburnus alburnus*), Minton and McLean (1982)

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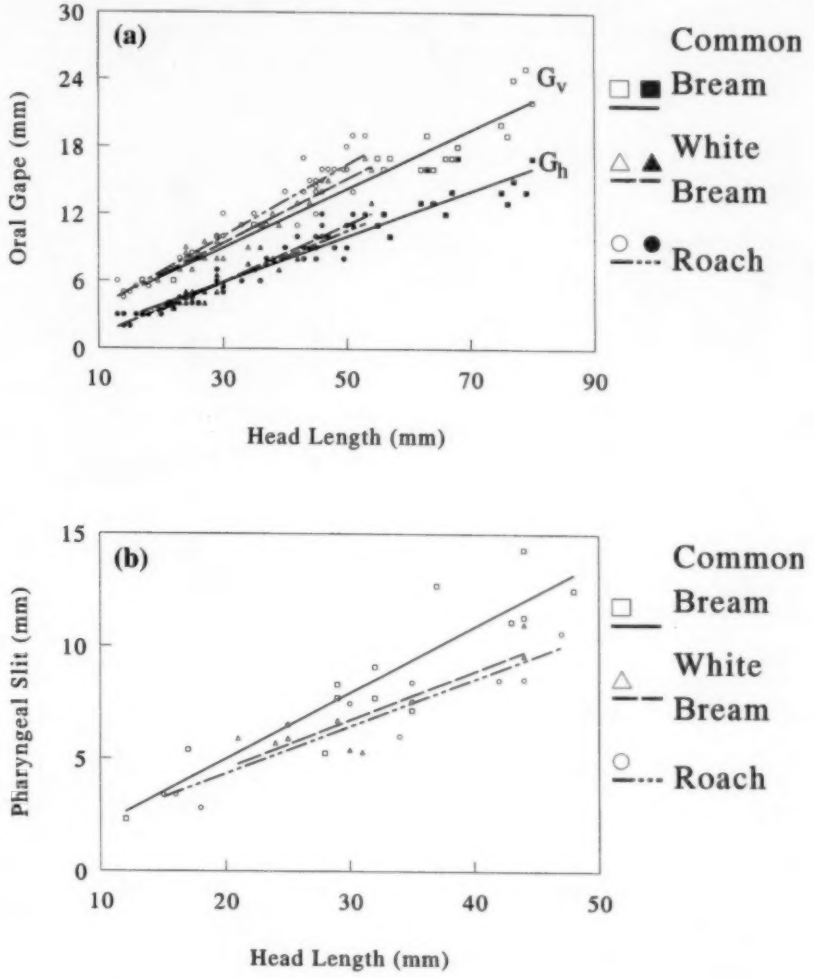
Fig. 8. Oral and pharyngeal dimensions for comparison with prey size (cf. Table 2). (*a*) Vertical oral gape (G_v, open symbols), horizontal oral gape (G_h, solid symbols), and their regression lines (with head length (HL) as independent variable). Common bream: $G_v = 1.096 + 0.263$ HL ($n = 18$, $R^2 = 0.88$), $G_h = -0.223 + 0.204$ HL ($n = 18$, $R^2 = 0.92$). White bream: $G_v = 0.752 + 0.288$ HL ($n = 26$, $R^2 = 0.93$), $G_h = -1.749 + 0.255$ HL ($n = 26$, $R^2 = 0.93$). Roach: $G_v = 0.398 + 0.320$ HL ($n = 42$, $R^2 = 0.90$), $G_h = -1.089 + 0.232$ HL ($n = 42$, $R^2 = 0.92$). (*b*) Pharyngeal slit (PS) and its regression lines. Common bream: $PS = -0.889 + 0.294$ HL ($n = 13$, $R^2 = 0.81$). White bream: $PS = 0.203 + 0.217$ HL ($n = 8$, $R^2 = 0.77$). Roach: $PS = 0.134 + 0.210$ HL ($n = 11$, $R^2 = 0.89$).



found a value of 3.8 for sauger (*Stizostedion canadense*), and Brett and Groves (1979) reported a value of 8.5 for sockeye salmon (*Oncorhynchus nerka*). If we assume (arbitrarily, but on the basis of these data) that for our species the average metabolic rate is 5 times R_s, FE has to be at least 5 to gain energy for growth and reproduction if the fish spend all of their time for feeding. Periods of nonfeeding will impose even higher demands on feeding efficiency. The average FE values for common bream range from 8 to 17 (Table 1), suggesting that this species would have to spend a large part of its time (29–63%) as handling time to be able to feed on zebra mussels profitably. White bream and roach have much higher average FE values (40–55 and 22–37, respectively) and therefore could gain more profit from zebra mussels than common bream.

The differences in FE among the three species are based on an equal average metabolic rate (eq. 5) and on an equal average digestibility (eq. 3). Welsh et al. (1988) showed that large errors can be introduced by using statistically correlated averages. If, for example, the lower mastication intensity of common bream (Fig. 4) resulted in a lower digestibility of the food, the profitability of zebra mussels would be even lower for this species, compared with the other two. However, if lower mastication intensity meant that common bream invested less energy, the profitability could increase. We expect that the differences in digestibility of zebra mussels are small among the three species of fish as soon as the shell is crushed, because of the high protein content of the prey and because the three species are closely related. This relatedness also justifies the

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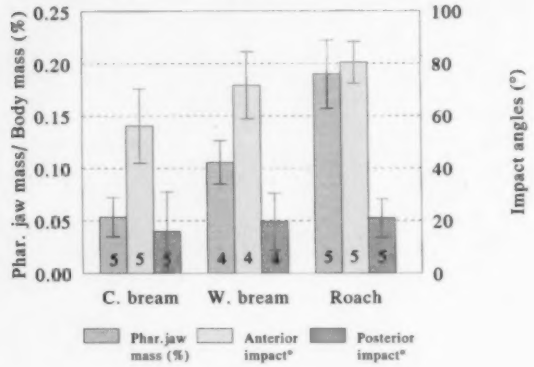
Fig. 9. Pharyngeal jaw mass as a percentage of body mass and the anterior and posterior impact angles of the pharyngeal teeth on the chewing pad. Total means and standard deviations are shown, as well as the number of measured fish (bottom of bars). Note that the pharyngeal jaw mass is smallest in common bream and largest in roach (cf. Fig. 1). The anterior impact angle is smallest in common bream and largest in roach. Posterior impact angles are about equal. Large pharyngeal jaw mass and anterior impact angles increase the ability to crush mollusc shells (see text).



use of the same equation for the standard metabolic rate. Differences in overall metabolic rate are difficult to establish, so this could be an important source of error. However, the differences between common bream on the one hand and white bream and roach on the other are so large that even with large errors, species efficiencies should remain distinct. The difference between white bream and roach may be affected by these errors and may partly explain why white bream has higher FE values than roach, although the structural parameters of roach appear to be better suited for molluscivory and roach eat more mollusks in the field (Rask 1989; Lammens et al. 1987, 1992).

In periods of lean food conditions trophic segregation appears among our three species (Lammens et al. 1987). At sizes smaller than 15 cm SL, they all feed mainly on copepods and cladocerans. At larger body sizes, however, white bream switches to chironomid larvae and pupae and zebra mussels, and roach switches to snails. Common bream does not feed on mollusks, but switches to chironomid larvae at 20–30 cm SL. One cause for this segregation may be the differences in feeding efficiency on zebra mussels, or, more generally speaking, the strength and size of shelled mollusks. Prey detection and foraging behavior could provide other causes, e.g., roach is a more actively swimming and visually oriented fish than the deep-bodied common bream and white bream, increasing its encounter rate with the prey (Lammens et al. 1987).

Rask (1989) reported that roach can feed successfully on zebra mussels, eating more mollusks than the other two species, suggesting that it is the better molluscivore. This may be explained by our conclusion that roach can process larger mussels than the other two species. When expressed as a percentage of SL, both the maximal and the optimal prey size are significantly smallest in common bream. The differences of the means between white bream and roach are not statistically significant. When comparing fish of the same body mass (M; Table 2), however, W_{max} and W_{opt} both increase from common bream to white bream to roach (e.g., for fish of 103–112 g, W_{max} increases from 2.3% of SL for common bream, to 3.6% for white bream, and to 4.2% for roach; W_{opt} increases from 0.98% of SL for common bream, to 2.8% for white bream, and to 3.6% for roach; cf. Table 2).

An optimal prey size of about 60% of the oral gape was described by Werner (1974) for sunfish, and by Prejs et al. (1990) for roach. We found such 60% values for W_{opt} in two of the three roach (60.3, 86, and 59.6% of G_h) and in two of the four white bream (48.1, 62.4, 58.3, and 80.5%). Common bream had much smaller optimal prey sizes (31.2, 20.0, and 38.9% of G_h). Using G_v, these percentages were even smaller. It is not clear whether these authors used the width or the length of the prey nor whether the horizontal or vertical oral gape was used. In our study, there is no direct relationship between the optimal prey size and the oral gape, since other structural parameters limit prey size (Table 2).

Feeding structures limiting prey size

To what extent are the differences in feeding efficiencies, W_{max} and W_{opt}, explained by species characters in feeding structures and their functioning? The structural parameters measured in roach appear to be better matched for eating (large) zebra mussels than those in common bream and white bream (Table 2). This means that the largest mussels that can be taken in by roach can also be processed, which corroborates the conclusion of Prejs et al. (1990). In common bream and white bream the dimensions of the oral gape and the pharyngeal slit do not limit W_{max}. The largest mussels these two species can pass through their oral gape are larger than W_{max} and therefore cannot be further processed and utilized. Such mussels were eventually rejected.

The parameters measured for the pharyngeal jaws (Fig. 9) clearly suggest the superiority of roach in crushing strong and large shells. The pharyngeal jaw mass/body mass ratio is a measure of the forces a jaw can exert (Sibbing 1991*b*). The largest forces can be applied by roach (mean ratio of 1.90×10^{-3}) and the smallest by common bream (mean ratio of 0.53×10^{-3}), while white bream is intermediate (mean ratio of 1.06×10^{-3}, cf. Fig. 9). The impact angle of the anterior pharyngeal teeth is largest in roach (mean of 80°), which reduces the bending forces on the tooth, another character indicating adaptation for crushing, suited for the diminution of hard and brittle materials like shells.

The higher frequencies and shorter durations of the masticatory strokes in common bream and white bream (Figs. 2, 4), as well as the X-ray movies, show that these fish repeatedly hammer the mussel, using impact loading instead of static loading. For such impact crushing, the stroke of the pharyngeal jaws needs to have a certain amplitude to gain velocity and kinetic energy. This amplitude requires free space within the chewing cavity, and therefore, in both common and white bream, impact crushing limits prey size (W_{max}). Roach clamps the mussel between the pharyngeal teeth and chewing pad (Fig. 3), exerting a large static force during a longer period (Figs. 2, 4). This powerful static crushing requires no free space within the chewing cavity and explains why roach is superior in crushing large and strong shells (Table 2).

In conclusion, in roach the dimensions of gape, pharyngeal slit, and chewing cavity more or less equally limit the maximal prey size (gape limited, cf. Zaret 1980), while the type and size

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Table 1. Coefficients a and b of the least-squares exponential regression of the handling times (T) versus prey width (W): $T = a \exp^{bW}$.

	SL (mm)	Mass (kg)	a	b	R^2	n	FE Mean ± SD	FE Range	W_{opt} (mm)	% of SL	W_{opt*} (mm)	% of SL
Common bream	155	0.040	0.820	1.614	0.75	19	7.93±3.28	3.42–13.35	2.15	1.4	1.44	0.93
	204	0.105	0.667	1.029	0.86	20	12.29±5.00	5.06–24.34	2.00	0.98	2.26	1.1
	298	0.408	1.085	0.473	0.85	15	17.37±3.47	11.42–24.34	5.80	1.9	4.91	1.7
White bream	128	0.038	1.104	0.735	0.78	12	40.18±14.01	28.22–75.69	2.60	2.0	3.16	2.5
	186	0.112	0.703	0.573	0.77	21	46.18±13.17	12.22–74.05	5.30	2.8	4.05	2.2
	235	0.249	0.943	0.368	0.66	18	55.13±18.53	30.18–86.59	6.70	2.9	6.31	2.7
	246	0.304	1.946	0.271	0.59	23	45.09±19.29	16.27–96.86	9.50	3.9	8.57	3.5
Roach	146	0.040	2.953	0.472	0.81	25	36.65±14.32	11.99–60.90	3.80	2.6	4.92	3.4
	207	0.103	3.530	0.360	0.64	29	31.36±15.25	14.42–77.34	7.40	3.6	6.45	3.1
	235	0.216	3.517	0.325	0.68	7	22.35±9.17	14.44–41.35	6.80	2.9	7.14	3.0

Note: For all regressions, $p < 0.001$, except for roach of 235 mm standard length ($p < 0.05$). Feeding efficiencies (FE) were calculated from the measured handling times. Actual optimal prey size (W_{opt}) is the W value at which the highest FE was recorded. Theoretical optimal prey size (W_{opt*}) was derived from the regression equations for the handling time (see eq. 11 and text). SL, standard length (mm); n, number of consumption events monitored.

Table 2. Sizes of fish and zebra mussel prey ((empirical) W_{max} and W_{opt}), and prey sizes as percentages of fish standard length (SL), vertical and horizontal oral gape (G_v and G_h), and pharyngeal slit (PS).

						Maximal prey data (W_{max})					Optimal prey data (W_{opt})				
	SL (mm)	M (kg)	G_h (mm)	G_v (mm)	PS (mm)	W_{max} (mm)	% of SL	% of G_h	% of G_v	% of PS	W_{opt} (mm)	% of SL	% of G_h	% of G_v	% of PS
Common bream	155	0.040	6.9	10.3	9.4	3.00	1.9	43.3	29.2	31.9	2.15	1.4	31.2	20.9	22.9
	204	0.105	10.0	14.2	13.8	4.70	2.3	47.1	33.0	34.0	2.00	0.98	20.0	14.1	14.5
	298	0.408	14.9	20.5	20.9	7.00	2.3	47.0	34.1	33.6	5.80	1.9	38.9	28.3	27.8
White bream	128	0.038	5.4	8.8	6.3	4.10	3.2	75.9	46.6	65.2	2.60	2.0	48.1	29.5	41.3
	186	0.112	8.5	12.3	8.9	6.70	3.6	79.2	54.7	75.3	5.30	2.8	62.4	43.1	59.6
	235	0.249	11.5	15.7	11.5	9.30	4.0	80.7	59.2	80.9	6.70	2.9	58.3	42.7	58.3
	246	0.304	11.8	16.0	11.7	9.70	3.9	82.3	60.6	82.8	9.50	3.9	80.5	59.4	81.2
Roach	146	0.040	6.3	10.6	6.9	5.40	3.7	85.4	50.8	78.7	3.80	2.6	60.3	35.8	62.3
	207	0.103	8.6	13.8	9.0	8.70	4.2	100.7	62.9	97.1	7.40	3.6	86.0	53.6	82.2
	235	0.216	11.4	17.7	11.5	10.30	4.4	90.2	58.3	89.7	6.80	2.9	59.6	38.4	59.1

of the masticatory force is the main restricting factor in white bream and common bream (chewing limited). Similarly, in the molluscivorous Caribbean hogfish, *Lachnolaimus maximus*, the maximal force that could be produced through the pharyngeal jaws was the limiting factor in crushing the shell, while the dimension of the chewing cavity was not (Wainwright 1987). In view of its pharyngeal jaw development it is likely that roach can utilize even harder shells of similar W_{max} than those of zebra mussel.

Conflicting demands for molluscivory and filter feeding

Why has common bream such weak pharyngeal jaws and an oversized mouth? In a multifunctional construction like the head of a fish, the size and shape of a structure affect the size and shape of other (adjacent) structures (Barel 1993). In the case of our three closely related cyprinid species this means that the large pharyngeal jaws and masticatory muscles of roach leave less space for other head structures, e.g., the branchial sieve. In a comparison of the three species Van den Berg et al. (1992) showed that the filtering area of the branchial sieve is approximately two times larger in common bream than in roach, while that of white bream is intermediate in size. Strong development of one functional component reduces the opportunities for another. In an ecological sense this means that fish that are more specialized for a certain food type inevitably lose some of their abilities to utilize other food resources. So, in the case of common bream, the large capacity for filtering zooplankton is not compatible with the efficient utilization of zebra mussels. In roach this is reversed, while white bream is intermediate for both structures (Lammens et al. 1987; Lammens and Hoogenboezem 1991). The large oral gape of common bream seems to be oversized when considering W_{max} but is a necessity in view of the large volumes of water that have to be taken in for profitable filter feeding (Sibbing 1991*a*).

Conclusions

(*i*) In feeding on zebra mussels, handling time and its associated standard metabolic energetic requirements dominate the total energetic costs (1–29% of the actual energy benefits from the mussel, E_m). Extra energetic costs for mechanical actions such as detaching and crushing the shell only require a fraction of the spent energy (0.01–0.15% of E_m) (Fig. 6). Thus, total handling time, including deglutition, is a good parameter for comparing feeding efficiency and is a profitable parameter to optimize.

(*ii*) Handling time increases with mussel size and is inversely related to fish size (Fig. 5). Handling time for a particular mussel size increases from white bream and roach towards common bream.

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(*iii*) Feeding efficiency for a particular mussel increases from common bream towards roach and white bream; for common bream it also increases with fish size (Fig. 7).

(*iv*) A comparison of energetic costs and benefits in feeding on zebra mussels suggests that profitability is possibly only marginal for common bream, whereas it allows growth and reproduction in white bream and roach.

(*v*) Maximal and optimal prey size are largest for roach and by far the smallest in common bream. In roach oral gape, pharyngeal slit and chewing cavity all match maximal prey size (Table 2). In common and white bream this is not the case.

(*vi*) Roach is superior in static crushing hard and brittle materials like zebra mussel shell. This is apparent from the structure and mass of its pharyngeal jaws (Figs. 1, 9) and from its low-frequency long masticatory strokes (Fig. 2). Common bream has the poorest abilities for crushing. The impact type of crushing of common and white bream limits the maximal mussel size that can be consumed by these species.

(*vii*) The extensive branchial sieve of common bream for filter feeding conflicts with constructional demands for pharyngeal crushing.

(*viii*) The different efficiencies of common bream, white bream, and roach in feeding on shelled mollusks of particular size and toughness could contribute to trophic segregation during periods of lean food conditions.

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Appendix

Symbols and notation.

Symbol	Definition	Units
AFE	Absolute feeding efficiency	—
E_{br}	Breaking energy	J
E_{cr}	Crushing energy	J
E_{ft}	Total energy costs of feeding	J
E_m	Actual energy benefits from mussel	J
E_{mt}	Total energy content of mussel	J
E_p	Pulling energy	J
E_s	Standard metabolic energy requirements during the feeding process from intake until deglutition	J
F_{br}	Breaking force	N
F_{cr}	Crushing force	N
F_p	Pulling force	N
FE	Empirical feeding efficiency	—
FE_*	Theoretical feeding efficiency	—
G_h	Horizontal oral gape dimension	mm
G_v	Vertical oral gape dimension	mm
H	Height of mussel	mm
HL	Head length	mm
L	Length of mussel	mm
M	Body mass of fish	kg
PS	Vertical limiting dimension of pharyngeal slit	mm
R_s	Standard metabolism	W
s_{br}	Deformation of material at breaking point	m
s_{cr}	Deformation of shell at breaking point	m
s_p	Deformation of byssus threads at breaking point	m
SL	Standard length	mm
T	Handling time	s
W	Width of mussel	mm
W_{max}	Maximal prey width	mm
W_{opt}	Optimal prey width	mm
W_{opt*}	Theoretical optimal prey width	mm

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Dynamics of radiolabelled methylmercury in crayfish (*Orconectes virilis*)

C.M. Headon, R.J. Hall, and G. Mierle

Abstract: A laboratory study was conducted to determine the assimilation efficiency of methyl[^{203}Hg]mercuric chloride ($CH_3{}^{203}HgCl$) from food by crayfish (*Orconectes virilis*) and the subsequent depuration rate of the assimilated $CH_3{}^{203}HgCl$. Mean assimilation efficiency of $CH_3{}^{203}HgCl$ from tadpoles was 96%. For the majority of the 50 experimental crayfish (three size-classes of males and females designated small, medium, and large), no significant loss of assimilated $CH_3{}^{203}HgCl$ was evident over the 166-day monitoring period. When a significant loss of $CH_3{}^{203}HgCl$ was detected, observed half-lives usually fell within 1000–2000 days (3–5 years). The high assimilation efficiency and slow depuration rate imply that crayfish can biomagnify CH_3Hg from their food.

Résumé : Nous avons effectué une étude en laboratoire pour mesurer l'efficacité d'assimilation du chlorure de méthyl[^{203}Hg]mercure ($CH_3{}^{203}HgCl$) présent dans la nourriture des écrevisses (*Orconectes virilis*) et le taux subséquent de dépuration du $CH_3{}^{203}HgCl$ assimilé. L'efficacité moyenne d'assimilation du $CH_3{}^{203}HgCl$ chez les têtards était de 96%. Chez la majorité des 50 écrevisses de l'expérience (trois classes de taille de mâles et de femelles désignées comme petite, moyenne et grande), on n'a observé sur la période d'étude de 166 jours aucune disparition significative du $CH_3{}^{203}HgCl$ assimilé. Quand une disparition significative du $CH_3{}^{203}HgCl$ était détectée, les demi-vies observées tombaient généralement entre 1000 et 2000 jours (3–5 ans). La forte efficience d'assimilation et le faible taux de dépuration permettent de penser que les écrevisses peuvent bioamplifier le CH_3Hg à partir de leur nourriture.
[Traduit par la Rédaction]

Introduction

Mercury (Hg) has become a growing concern, especially in lakes remote from known Hg point sources. A number of pristine lakes in Ontario contain predatory fish with Hg levels exceeding the Canadian 0.5 $\mu g \cdot g$ wet mass^{-1} upper limit for safe consumption (Suns et al. 1987; Ontario Ministry of the Environment and Energy and Ontario Ministry of Natural Resources 1993). Fish accumulate Hg from water as it passes over the gills and from ingested food (Norstrom et al. 1976; Phillips and Buhler 1978; Rodgers and Qadri 1982; Rodgers et al. 1987). Practically all (>95%) of the total Hg in fish muscle tissue is in the form of methylmercury (CH_3Hg) (Grieb et al. 1990; Bloom 1992). The ability of CH_3Hg to bioaccumulate to high levels relative to the inorganic form seems to be related firstly to the relative difference in excretion rate (CH_3Hg is excreted more slowly than the inorganic form (Järvenpää et al. 1970; Kramer and Neidhart 1975; Ruohtula and Miettinen 1975; Pentreath 1976; Sharpe et al. 1977)) and secondly to a more efficient assimilation from food and water (Olson et al. 1973; Kramer and Neidhart 1975; Pentreath 1976; deFreitas 1977; Boudou and Ribeyre 1985).

Crayfish could be a significant vector for CH_3Hg to fish and wildlife. They are a significant component of the organisms associated with lake and stream sediments, sometimes occurring in large numbers (Momot and Gowing 1977; Momot 1978; Reid and David 1990). Crayfish also belong to several trophic levels and consume a wide variety of food types, such as algae, detritus, vegetation, benthic invertebrates, and fish remains (Momot et al. 1978; Capelli 1980; Goddard 1988; Hanson et al. 1990). In turn, crayfish are an important food source for a number of fish species (Fedoruk 1966; Reid 1972; Stein 1977), fish-eating birds (Hoffman and Curnow 1979), and mammals such as mink (*Mustela vison*; Linscombe et al. 1982), river otter (*Lutra canadensis*; Toweill and Tabor 1982), and raccoon (*Procyon lotor*; Kaufmann 1982). Further, Hg bioaccumulation has been well demonstrated in these crustaceans (even in systems remote from known direct sources of Hg) through correlations between Hg concentration and size (Vermeer 1972; Armstrong and Hamilton 1973; Stinson and Eaton 1983; Allard and Stokes 1989).

To understand the role of crayfish in mediating CH_3Hg flux, information on the rates of CH_3Hg uptake and depuration is required. Three published studies have examined Hg depuration in crayfish (Miettinen et al. 1969; Hamilton 1972; Wright et al. 1991). However, there are substantial differences among the results. Wright et al. (1991) and Miettinen et al. (1969) reported that crayfish were able to eliminate significant quantities of assimilated $CH_3{}^{203}Hg$, with calculated biological half-lives of 2 and 144 days, respectively. In contrast, Hamilton (1972) observed that crayfish that contained naturally high levels of Hg were unable to lose even small amounts. Since knowledge of the rate at which an organism can depurate CH_3Hg is essential for modelling CH_3Hg dynamics, the published discrepancies have to be resolved.

Our study had three objectives: (*i*) to determine the assimilation efficiency of CH_3Hg from food by *Orconectes virilis*, the most widely distributed Canadian crayfish species (Crocker

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Table 1. Water chemistry of aquarium water throughout the depuration period.

Chemical parameter	*n*	Mean ± SD	Range
pH	48	6.81±0.61	5.44 to 7.50
Alkalinity ($mg \cdot L^{-1}$)[a]	48	9.44±7.37	–0.07 to 23.92
Conductivity ($\mu S \cdot cm^{-1}$)	62	102.69±6.42	89.9 to 115.4
Ammonium (as N) ($\mu g \cdot L^{-1}$)	48	107±181	3 to 810[b]
$NO_2 + NO_3$ (as N) ($\mu g \cdot L^{-1}$)	48	2706±1675	10 to 5100
Dissolved oxygen ($mg \cdot L^{-1}$)	48	8.7±0.85	7.0 to 10.9
DIC ($mg \cdot L^{-1}$)	48	2.40±1.72	0.35 to 5.81
DOC ($mg \cdot L^{-1}$)	48	0.5±0.3	0.1 to 1.4[b]
Hardness (as $CaCO_3$) ($mg \cdot L^{-1}$)	48	22.91±3.61	18.00 to 32.00
Ca ($mg \cdot L^{-1}$)	48	6.07±1.10	4.59 to 8.40
Cd ($\mu g \cdot L^{-1}$)	2		<0.001 to 0.020
Cu ($\mu g \cdot L^{-1}$)	2		0.137 to 0.241
Zn ($\mu g \cdot L^{-1}$)	2		1.707 to 2.415
Hg ($ng \cdot L^{-1}$)	1	<0.2[c]	

[a]Total inflection point alkalinity.

[b]The minimum value of the range was a measurable trace amount and should be interpreted with caution.

[c]Duplicate sample taken 2 months prior to the end of the study.

and Barr 1968; Momot 1988), (*ii*) to determine the biological half-life of assimilated CH_3Hg for *O. virilis*, and (*iii*) to determine the effect of *O. virilis* size and sex on the clearance of assimilated CH_3Hg.

Materials and methods

Crayfish collection and maintenance

Crayfish were collected from August through October 1991, from Blue Chalk Lake, a small circumneutral drainage lake located on the Precambrian Shield of south-central Ontario. Crayfish were identified to species and sexed according to Crocker and Barr (1968). Abdominal flexor muscles from 11 of the collected crayfish were analyzed for total Hg to determine background levels. Concentrations ranged from 0.022 to 0.055 $\mu g \cdot g$ wet $mass^{-1}$ for carapace lengths (CPL) ranging from 20.9 to 34.7 mm.

All laboratory work was conducted at the Dorset Research Centre, Ontario Ministry of the Environment and Energy, Dorset, Ontario. To ensure that no ^{203}Hg was released into the natural environment, a continuously recirculating water system was required for the study. Water flowed through one of two Hytrex II polypropylene particulate filters, followed by one of two activated charcoal filters. The filtered water was also treated with an ultraviolet light sterilizer before flowing into each glass aquarium (38-L capacity) at a rate of 0.5–1 $L \cdot min^{-1}$. Aquarium water flowed out of each tank via polyvinylchloride (PVC) piping into a 42-L high-density polyethylene reservoir, which was emptied daily and refilled with filtered lake water.

Four crayfish were assigned to each aquarium and were separated from each other with perforated PVC partitions. Each crayfish was provided a piece of PVC piping for shelter. Water was continuously aerated by air stones placed one per tank. Temperature of tank water ranged from 17 to 21°C. Crayfish were maintained in a constant lighting regime of 16 h light : 8 h darkness (summer lighting). Large and medium-sized crayfish (sizes defined below) were fed minnows daily at 3–5% body mass. Small crayfish were provided a daily ration at approximately 10% body mass. This feeding regime was adequate as there was usually a small amount of unconsumed food observed the following day. Minnows had a total Hg concentration of 0.121 ± 0.023 $\mu g \cdot g$ wet $mass^{-1}$ (mean ± SD) ($n = 8$).

Water chemistry for the recirculating water system was monitored regularly (Table 1). Initially, water pH was close to 7. However, pH dropped and remained fairly low (pH 5.4–5.9) for 2 weeks about a month into the depuration monitoring period. Water pH was gradually raised with additions of 10 mM NaOH and monitored closely until the termination of the study. During this period, pH ranged between 6.5 and 7.5.

Experimental design

Fifty *O. virilis* (26 males and 24 females) were exposed to methyl[^{203}Hg]mercuric chloride ($CH_3{}^{203}HgCl$). Twenty crayfish were classified as large (CPL 32.7–39.7 mm; mass 8.6–15.0 g), 21 as medium (CPL 27.0–30.4 mm; mass 4.7–8.1 g), and 9 as small (CPL 17.3–24.9 mm; mass 1.0–4.0 g). The sample size for small crayfish was low because there was considerable mortality prior to the commencement of the study. Seven additional crayfish acted as handling controls, as well as indicators of ^{203}Hg recirculation within the aquaria. These crayfish were treated in exactly the same manner as the test crayfish, except they were not fed food labelled with $CH_3{}^{203}HgCl$. Controls were dispersed among the $CH_3{}^{203}HgCl$-exposed crayfish within the aquaria.

Isotope preparation

Methyl[^{203}Hg]mercuric chloride (specific activity 49.15 $mCi \cdot g^{-1}$ of Hg) was purchased from Amersham (Arlington Heights, Ill.) as a solid. Upon arrival, the $CH_3{}^{203}HgCl$ was dissolved in an aqueous solution of 0.05 M HCl, resulting in a final concentration of 25 $\mu Ci \cdot mL^{-1}$. At this concentration, radiation-induced chemical breakdown is very slow and, therefore, the level of ^{203}Hg-labelled impurities remains low (Sharpe et al. 1977). The chemical purity of the aqueous $CH_3{}^{203}HgCl$ solution was tested (Headon 1994). Calculated mean percent organic Hg for the purchased radioisotope was 94 ± 2% ($n = 6$).

Prior to dosing crayfish with $CH_3{}^{203}HgCl$, the specific activity of the primary stock solution was determined. On the basis of the measured total Hg concentration (Headon 1994) and the activity reported by Amersham, the specific activity of the purchased isotope was calculated to be 70.01 $mCi \cdot g^{-1}$, a value higher than that reported by Amersham. When determining the dose of stable Hg that each crayfish had received, the new calculated specific activity was used. The activity of the primary stock solution (25 $\mu Ci \cdot mL^{-1}$) was taken to be correct and counting efficiencies were determined for all standards on the basis of this assumption.

Radioactivity measurements

Radioactivity was measured using a flat sodium iodide (NaI) crystal (6 cm diameter) connected to a multichannel analyzer. The gamma peak for ^{203}Hg is at 279 keV. The width of the window on the spectrum was adjusted visually to include all of the peak except for the smallest portion of the tails. At least 4000 – 10 000 counts were collected (usually in 100 s) to keep the statistical counting error less than 1.6%. During the initial postexposure period, crayfish were counted for 100 s and often 10 000 – 40 000 counts were measured. Towards the end of the study, 400 s (and occasionally 1000 s) were required to obtain 4000 counts. Variation in counting geometry was assessed by making at least three consecutive counts for each crustacean. However, if the crayfish had to be counted for long periods, only two consecutive counts were made.

Appropriate standards and background measurements were counted before and periodically throughout each counting session. All counts were corrected for background readings (90–120 counts/min (cpm)), converted to disintegrations per minute (dpm) using an appropriate counting efficiency, and corrected for radiological decay. ^{203}Hg has a half-life of 46.6 days (CRC Press Inc. 1983).

Whole-body counts of live crayfish were measured by placing each crayfish ventral side down in a PCV pipe chamber. The chamber was placed on the NaI crystal with the midpoint of the animal over the

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centre of the crystal. To standardize whole-body crayfish counts, "phantom" crayfish were prepared. Crayfish of different sizes were sacrificed, preserved in 70% ethanol, drained on towelling, and then injected with a known aliquot of $CH_3^{203}HgCl$. The radioisotope was allocated to different body regions (abdomen, cephalothorax, and left and right chelae) on the basis of their proportions of the total crayfish mass. Whole-body counts of the phantoms were made in the same manner as for the live crayfish. Counting efficiencies of phantoms (actual cpm divided by theoretical dpm) ranged from 3.45 to 6.71% with CPLs ranging from 35.1 to 19.5 mm ($n = 12$). Crayfish phantoms were not counted every counting session. However, a 5-mL standard in a capped Teflon vial was counted regularly to monitor fluctuations in sensitivity of the counting system, which fluctuated <5% over the duration of the study. Additional standards were also counted regularly. For each counting session, predetermined phantom counting efficiencies were appropriately adjusted on the basis of the counting efficiency of the above 5-mL standard.

Changes in counting efficiency of crayfish owing to growth had to be considered. Nearly all of the experimental crayfish moulted at least once, many twice, while being monitored for depuration. The average CPL growth increment per moult was 2.2 mm or approximately 4.4 mm in total length. To estimate the change in crayfish counting efficiency with size, counting efficiencies for the 12 phantoms were regressed against $\log_{10}$ CPL. The regression equation used to correct for a change in counting efficiency when a crayfish increased in size after ecdysis was

$$\text{efficiency (\%)} = -11.912 \log_{10} \text{CPL (mm)} + 22.033, \quad r^2 = 0.976,\ p < 0.01$$

Forty-nine days into the experiment there was concern that some crayfish were moving significantly inside the counting chambers. Therefore, all experimental crayfish from that point on were wrapped in cotton gauze. Movement was restricted substantially, resulting in considerable improvement in the reproducibility of consecutive counts.

Assimilation efficiency of $CH_3^{203}HgCl$

After a minimum 2-week acclimation period in the aquaria, assimilation efficiency was estimated by feeding crayfish tadpoles labelled with a known amount of $CH_3^{203}HgCl$. Details of the technique are provided in Headon (1994). Briefly, dead tadpoles (frozen and thawed) were individually incubated for 1.5 h with a known amount of $CH_3^{203}HgCl$ in a small volume of water. The quantity of $CH_3^{203}HgCl$ used was such that the whole-body concentration of CH_3Hg above background in the crayfish would be approximately 1 μg·g wet mass^{-1}. After a brief rinse, tadpoles, one per animal, were fed to crayfish starved during the previous 48 h. On average 79 ± 6% of the radioisotope was adsorbed by the tadpole. Feeding was carried out individually in 1000-mL sheltered glass beakers in which the crayfish had been acclimated for 2–9 days (mean 5 days). Immediately upon consumption of the labelled tadpole, usually within 10–15 min, the crayfish was measured for radioactivity. The amount of isotope in the water, in particulates, and adsorbed to the beaker was also estimated and, by difference from the amount in the tadpole, the amount ingested was determined.

Immediately after feeding, the crayfish was placed in a clean beaker of water for 24 h to collect faecal material. By repeating the above measurements of radioisotope in water, in particulates, and adsorbed to the beaker, the amount assimilated was estimated by difference from the amount ingested.

Depuration of assimilated $CH_3^{203}HgCl$

Live whole-body counts for each study crayfish were made two to three times weekly, over 166 days. During this time, crayfish were kept in the aquaria. When a crayfish was observed to have died, it was removed from its aquarium and homogenized, and the homogenate was measured for radioactivity. Likewise, shed exuvia (exoskeleton left behind after ecdysis) were removed from the water, homogenized, and counted.

To verify the depuration results calculated from live whole-body counts, 13 crayfish were sacrificed (between days 124 and 166) by freezing them for a period of 24–48 h. The crayfish were individually homogenized to produce samples with well-defined counting geometries. Sacrificing crayfish ensured that there was not a loss of radioisotope resulting from decay of the crustacean after death.

Live whole-body counts taken throughout the depuration phase of the study were corrected for radiological decay back to day 0, the day of consumption of the $CH_3^{203}HgCl$-labelled tadpole. For each crayfish, the natural logarithm of Hg content (corrected dpm) was plotted against time in days. Data were fitted to a straight line with a linear least squares regression program containing a lack of fit test written for the personal computer.

Results

Ingestion and assimilation of $CH_3^{203}HgCl$

On average 54 ± 25% (range 5–88%) of the $CH_3^{203}HgCl$ in the tadpoles was ingested. The portion and variability ingested reflect crayfish eating habits: they tear and shred their food before it enters the oesophagus, which results in a considerable portion of the food being left as debris. The quantity of radioisotope associated with collected food particles averaged 27% and ranged from 3 to 80% of the total isotope provided. The amount recovered in filtered water accounted for 18% (range 5–38%).

With the exception of a few outliers (see the section below on atypical depuration patterns), the assimilation efficiency of $CH_3^{203}HgCl$ from tadpoles was uniformly high (96 ± 2%, $n = 43$) across size-classes and sex. All of the unassimilated $CH_3^{203}HgCl$ was recovered in particulate faecal matter. No $CH_3^{203}HgCl$ was detected in the water, but because of its low counting efficiency (2.9%), a small amount may have been undetected. At most, however, the assimilation efficiency would be overestimated by 2% from undetected $CH_3^{203}HgCl$. Some of the crayfish ($n = 21$) were kept in beakers for an additional 24 h to test for delayed gut evacuation. On average, only 0.9 ± 0.7% of the initial amount of radioisotope consumed was detected in faecal material extruded between 24 and 48 h postexposure.

From the quantity of radioisotope assimilated, the resulting concentration of CH_3Hg above background in the crayfish body could be estimated. The estimated CH_3Hg concentrations (μg·g wet mass^{-1}) for the three size-classes were as follows: small, 1.41 ± 1.06 (range 0.14–3.02); medium, 0.97 ± 0.50 (range 0.19–1.85); and large, 1.11 ± 0.55 (range 0.20–1.92).

Depuration of $CH_3^{203}HgCl$

Although exuvia account for a large portion of the wet mass of crayfish (mean 52%), very little $CH_3^{203}Hg$ was eliminated by this route. In fact, for the majority of crayfish, detectable levels of $CH_3^{203}Hg$ were not measurable in shed exuvium. Crayfish that had trace amounts of isotope in their exuvium had moulted within 4 weeks of consumption of the radiolabelled tadpole. With the exception of two outliers (see the section below on atypical depuration patterns), detectable amounts of $CH_3^{203}Hg$ in exuvia accounted for only 0.3–1.4% of the assimilated isotope.

The most conspicuous feature of the depuration results is the very small change in ^{203}Hg content over most of the duration

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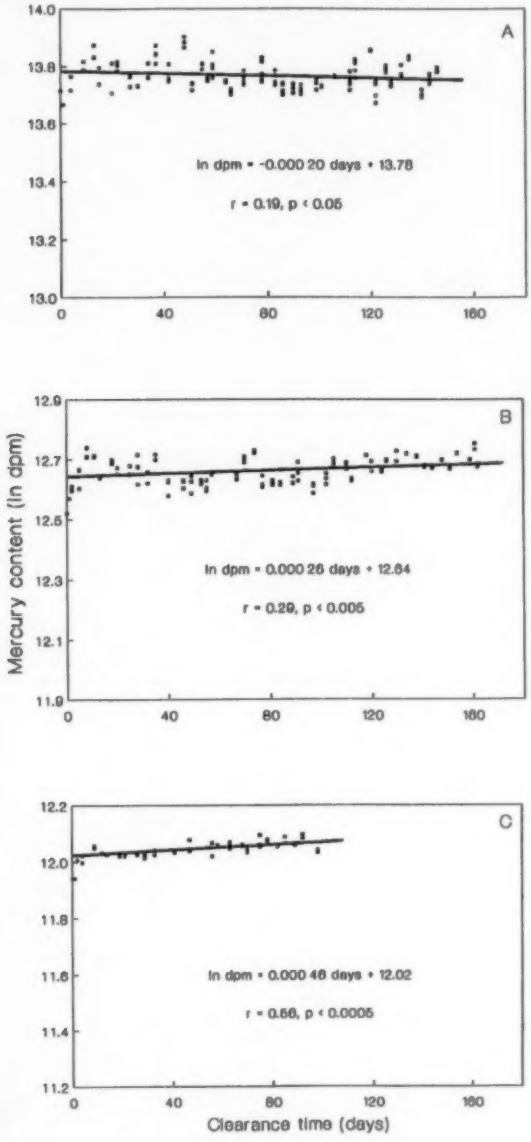
Fig. 1. Whole-body clearance of ^{203}Hg-labelled methylmercury over time. (A) Large female. (B) Medium male. (C) Small male.


A
ln dpm = -0.000 20 days + 13.78
r = 0.19, p < 0.05
B
ln dpm = 0.000 26 days + 12.64
r = 0.29, p < 0.005
C
ln dpm = 0.000 46 days + 12.02
r = 0.66, p < 0.0005
Mercury content (ln dpm)
Clearance time (days)

of the study (Fig. 1). These results clearly indicate that very little, if any, of the assimilated $CH_3^{203}HgCl$ was lost by the crayfish during the experiment. Two additional patterns, both artifacts, are evident on close examination of the data. Firstly, radioisotope counts often increased during the first few days after exposure. We expected to see a slight decrease during this period as the $CH_3^{203}Hg$ distributed through the body and changed the counting efficiency. However, crayfish were often observed raising their cephalothorax off the counting chamber floor. This action would increase the distance of the gastric mill to the crystal relative to the abdomen. Because initially the $CH_3^{203}HgCl$ would be located primarily in the gastric mill, this action would lower the counting efficiency relative to later periods when the isotope was distributed internally. The second artifact was an improvement in counting precision after day 49 (Fig. 2), a result of wrapping crayfish in gauze to restrict movement.

To determine the appropriateness of the single-compartment depuration model, the natural logarithm of the radioisotope decay rate was regressed against time, and the *F* statistic for lack of fit was used for testing the hypothesis. The initial changes in radioisotope counts, several atypical crayfish (see below), and three medium crayfish, which had been exposed to the ^{203}Hg-labelled food for 1.5–2 h, were excluded in this analysis. This test statistic was significant ($\alpha = 0.05$) for all but five crayfish, which suggests that the model was not appropriate. However, plots of the data suggest that the lack of fit was due to day to day variations in counting efficiency rather than nonlinear trends in the data. The lack of fit was evident only when the counting variation within a session was kept very low by wrapping the crayfish in gauze (Fig. 2).

The half-lives implied by the regressions are summarized in Table 2. If the slope of the regression was significantly different from zero ($\alpha = 0.05$), then a biological half-life was calculated. Overall, there was no apparent difference in $CH_3^{203}Hg$ elimination rate among size-classes or between sexes within each size-class. It should be noted that four of the small crustaceans died within a month after exposure. Half-lives reported for these crayfish have to be analyzed with caution because ^{203}Hg content data for only a few days were used in the regression analyses.

For a number of crayfish, negative biological half-lives (positive regression slopes) were obtained, a theoretical impossibility unless there was uptake of ^{203}Hg depurated from others. However, detectable levels of radioactivity were never observed for the seven control animals. The positive slopes are most likely an artifact of a small, unintentional increase over time in counting efficiency owing to subtle changes in how crayfish were wrapped or, perhaps, a small overcorrection in the adjustment of counting efficiency when they moulted. Another possibility is an increase in counting efficiency owing to redistribution of isotope during the experiment. However, analysis of tissues from six of the crayfish did not indicate any variation with time from exposure (Headon 1994). Whatever the cause, the results imply that the estimates of $CH_3^{203}Hg$ depuration are somewhat biased towards underestimation of loss.

To avoid the bias of the live whole-body counts, 13 crayfish were killed, homogenized, and counted using a well-defined, carefully calibrated counting geometry. Compared with the initial amount assimilated, these crayfish retained 86–108% of the $CH_3^{203}Hg$ with a mean of 94% (Table 3). Assuming a single-compartment depuration model, 94% retention over 153 days (the mean depuration period) implies a biological half-life of 1768 days (4.8 years). For 10 crayfish that had died in the aquaria during the experiment, little or no loss of radioisotope was suspected through decay of the organisms from time of death until removal from the water. The percentages of $CH_3^{203}Hg$ retained by these crayfish, which had died between day 13 and 155 (mean 101 days), were very similar to those retained by the sacrificed animals (Headon 1994). The pooled mean percentage of $CH_3^{203}Hg$ retained was 93 ± 6% (range 85–108%, $n = 23$).

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Fig. 2. Relationship between whole-body clearance of ^{203}Hg-labelled methylmercury and time for a large female crayfish. Within-day repeat measurements are connected by a vertical line. (A) Before crayfish was wrapped in cotton gauze. Lack of fit F statistic was not significant at $\alpha = 0.05$ ($F = 0.75$). (B) Crayfish wrapped in cotton gauze. Lack of fit F statistic was significant ($F = 10.3$).

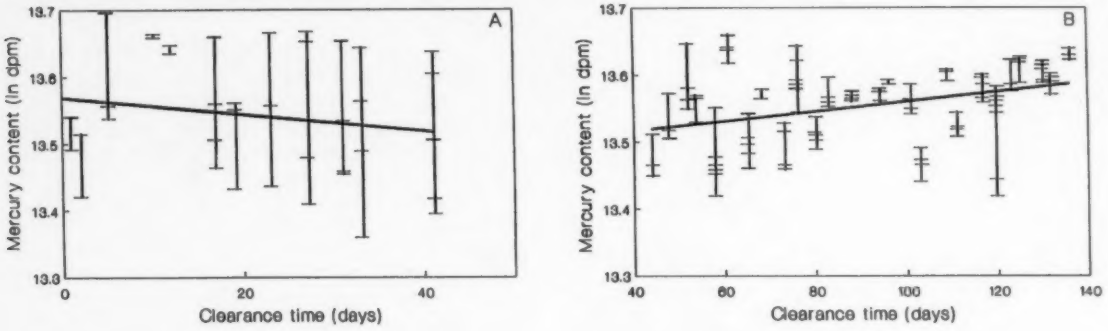

Table 2. Mean biological half-lives (with range given in parentheses) of assimilated $CH_3{}^{203}Hg$ and experimental duration for small, medium, and large crayfish.

	No loss[a]		Significant loss			Positive slope		
Size-class	*n*	Duration (days)	*n*	Half-life (days)	Duration (days)	*n*	Half-life (days)	Duration (days)
Small	7	61 (10–150)	0			1	–1498	98
Medium	5	109 (46–166)	5	1300 (541–1923)	114 (94–157)	6	–1532 (–514 to –2700)	148 (126–162)
Large	7	95 (27–129)	2	(1168–3409)	(96–146)	7	–1713 (–1302 to –2020)	142 (106–165)

[a]No significant loss at $\alpha = 0.05$.

Atypical depuration patterns

Six of the experimental organisms had uncharacteristic depuration curves relative to the rest of the study animals. There were two distinct components to each of these depuration plots. The first component exhibited an initial rapid drop in ^{203}Hg content with estimated half-lives ranging from 2 to 17 days. The second component of the curve had a slope of almost zero (Fig. 3). Two of these crayfish moulted shortly after assimilation of the radioisotope (2 and 6 days). Shed exuvia contained unusually high levels of ^{203}Hg, accounting for 7 and 19% of the ingested radioisotope. No additional ^{203}Hg was detected in exuvia from subsequent moults.

Five of the above six crayfish were in contact with the labelled food for unusually long periods of time (30–360 min) because they would not initially consume the tadpole. The sixth was exposed only for 10 min, but it was a very messy eater. The circumstances and the unusual amount of isotope on the exuvia suggest that $CH_3{}^{203}Hg$ adsorbed to their exoskeletons, either from the water ($CH_3{}^{203}Hg$ leached from the tadpole) or from direct contact with the labelled food, and that the rapid depuration component of these crayfish was due to surface desorption.

Discussion

Assimilation efficiency

The mean assimilation efficiency of $CH_3{}^{203}HgCl$ from tadpoles by *O. virilis* was 96%. This efficiency is somewhat higher than values noted for a number of fish species, which range from 70 to 90% (deFreitas 1977; Sharpe et al. 1977; Phillips and Buhler 1978; Rodgers and Beamish 1982; Boudou and Ribeyre 1985). Assimilation efficiencies similar to those for fish were also shown for the amphipod *Hyallela azteca* (Lloyd 1979) and the water flea *Daphnia magna* (Trudel 1980) (values for the invertebrates ranged from 60 to 90%).

Table 3. Mean (±SE) and range of $CH_3{}^{203}Hg$ quantities retained by crayfish over the experimental duration relative to quantities assimilated (expressed as percentages).

		Percent retained		Duration (days)	
Size-class	*n*	Mean	Range	Mean	Range
Small	2		87–108		150–161
Medium	8	91±2	86–100	157±4	135–166
Large	3	100±2	96–103	143±12	124–165

Note: Retained values are for sacrificed crayfish.

There are a number of factors that could have affected the assimilation efficiency of CH_3Hg. The type and quantity of food consumed may be important. Experimental crayfish were first starved for 48 h and fed only one tadpole. In addition, had the tadpoles in this study been labelled with $CH_3{}^{203}Hg$ more naturally, a lower assimilation efficiency may have been obtained. Most CH_3Hg assimilation efficiency studies have been conducted using commercial diets with added CH_3Hg (deFreitas 1977; Sharpe et al. 1977; Phillips and Buhler 1978; Rodgers and Beamish 1982) or prey injected with CH_3Hg (deFreitas 1977). However, Phillips and Gregory (1979) fed northern pike (*Esox lucius*) fish that were naturally contaminated with CH_3Hg and observed that the assimilation efficiency was only

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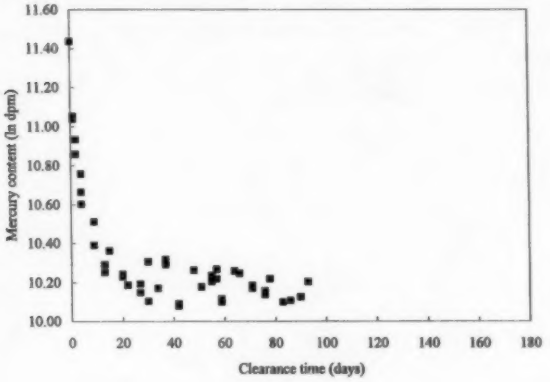
Fig. 3. Whole-body clearance of ^{203}Hg-labelled methylmercury over time for a large male crayfish that had been exposed to a contaminated tadpole for 90 min.



19%. They hypothesized that CH_3Hg accumulated naturally may be associated with nondigestible parts and, therefore, not assimilated to the same extent as CH_3Hg obtained from food where the CH_3Hg is loosely bound.

Tadpoles had to be dosed with high levels of $CH_3{}^{203}HgCl$ because of the short half-life of ^{203}Hg and the fairly low specific activity of the purchased isotope. For example, a 6.0-g crayfish would have been fed a tadpole with a CH_3Hg concentration of approximately 100 μg·g wet mass^{-1}. In nature, when foods with much lower CH_3Hg concentrations are consumed, the assimilation efficiency of CH_3Hg may be somewhat different from the value determined in this study. However, Rodgers and Beamish (1982) reported no difference in assimilation efficiencies of ^{203}Hg-labelled CH_3Hg (range 70–80%) for rainbow trout (*Oncorhynchus mykiss*) fed diets containing <0.1, 25, and 75 μg·g^{-1} of CH_3Hg for up to 8 weeks.

Methylmercury depuration

Experimental results revealed that significant quantities of assimilated $CH_3{}^{203}Hg$ were not eliminated with the shedding of the exoskeleton during ecdysis. Similarly, on the basis of the results of a study that looked at $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ dynamics in the marine shrimp, *Lysmata seticaudata*, shrimp moults account for only 2% of the whole-body Hg burden (Fowler et al. 1978). A survey of total Hg levels in adult *O. virilis* collected from six remote lakes revealed that of all the tissues analyzed, carapace (exoskeleton covering the cephalothorax) had the lowest Hg concentration (0.009–0.014 μg·g wet mass^{-1}) (C.M. Headon, unpublished data).

No significant loss of assimilated $CH_3{}^{203}HgCl$ was observed for the majority of experimental crayfish. When a significant loss of $CH_3{}^{203}Hg$ was detected, calculated biological half-lives usually fell within 1000–2000 days (3–5 years). Methylmercury depuration rates reported in the literature for a number of aquatic organisms are quite varied (Table 4).

As mentioned earlier, three other studies have also examined Hg depuration in crayfish. Miettinen et al. (1969) administered $CH_3{}^{203}HgNO_3$ orally into the gastric mill of 16 European crayfish (*Astacus fluviatilis*) and observed a mean biological half-life of 144 days. These crayfish were only monitored for 14–63 days (Miettinen et al. 1969). The biological half-life of $CH_3{}^{203}HgNO_3$ injected in the abdominal muscle of one crayfish was twice as long (297 days) (Miettinen et al. 1969). Wright et al. (1991) obtained substantially different CH_3Hg depuration results for *Orconectes propinquus*. For a 2-week period, Wright et al. (1991) fed experimental crayfish dry cat food labelled with $CH_3{}^{203}HgCl$. After a 3-week depuration period, nearly all of the accumulated $CH_3{}^{203}Hg$ in the abdominal muscle was eliminated (Wright et al. 1991). The fact that ^{203}Hg was almost completely lost from the abdominal muscle after 3 weeks of depuration is surprising. For this study, abdominal muscle was found to preferentially concentrate the ^{203}Hg-labelled radioisotope, even after 148 days of depuration (Headon 1994).

Table 4. Examples of biological half-lives of assimilated CH_3Hg recorded in the literature for a number of aquatic organisms.

	Half-life (days)	Reference
Rainbow trout (*Oncorhynchus mykiss*)	204–516	Ruohtula and Miettinen 1975
	55–134	Rodgers and Beamish 1982
Goldfish (*Carassius auratus*)	35–173	Sharpe et al. 1977
Pike (*Esox lucius*)	750	Järvenpää et al. 1970
Flounder (*Pleuronectes flesus*)	780	Järvenpää et al. 1970
Eel (*Anguilla vulgaris*)	910	Järvenpää et al. 1970
Plaice (*Pleuronectes platessa*)	163	Pentreath 1976
Mosquitofish (*Gambusia affinis*)	∞	Huckabee et al. 1975
Daphnia pulex	3.2	Huckabee et al. 1975
Daphnia magna	5	Trudel 1980
Hyalella azteca	40	Lloyd 1979
Marine shrimp (*Lysmata seticaudata*)	529	Fowler et al. 1978
Marine crab (*Carcinus maenas*)	400	Miettinen et al. 1972
Mollusc (*Tapes decussatus*)	481	Miettinen et al. 1972
Mollusc (*Mytilus galloprovincialis*)	1000	Miettinen et al. 1972
In situ	63	Fowler et al. 1978
Laboratory	377	Fowler et al. 1978

It is uncertain why the results obtained by Wright et al. (1991) differ so much from the results of the present study. One possibility is that Wright et al. (1991) used artificial lake water with relatively high concentrations of major ions and ethylenediaminetetraacetic acid (EDTA). Also, concentrations of "free" CH_3Hg may have been much lower in the water of their experiments.

In contrast to Miettinen et al. (1969) and Wright et al. (1991), Hamilton (1972) utilized crayfish that had been contaminated naturally with Hg. Adult *O. virilis* were collected from the Hg-contaminated Clay Lake, northwestern Ontario, placed in holding containers containing water and sediment uncontaminated with Hg, and fed fish with low Hg levels (0.13 μg·g wet mass^{-1}) (Hamilton 1972). Crayfish initially had a total Hg concentration in the abdominal muscle of 6.56 μg·g^{-1} wet mass. After an 83-day monitoring period, Hamilton (1972) concluded that ". . . crayfish are essentially unable to excrete even small amounts of mercury."

For the present study, a difference in the fractional clearance rate of $CH_3{}^{203}Hg$ among size-classes could not be

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detected. However, fish size has been shown to be an important factor controlling CH_3Hg depuration (Sharpe et al. 1977). Methylmercury was cleared from tissues of small goldfish (*Carassius auratus*) at a faster rate than from larger fish (Sharpe et al. 1977). When the logarithm of fractional clearance rate for CH_3Hg was plotted against the logarithm of fish mass, there was a significant negative correlation defined by the equation

$$K_{cl} = 0.029\ W^{-0.58}$$

where K_{cl} is the fractional clearance rate (day^{-1}) and W is the fish wet mass (g) (Sharpe et al. 1977).

The fractional clearance rates of assimilated CH_3Hg determined in this study for crayfish do not fit the above relationship. On the basis of the equation proposed by Sharpe et al. (1977), small crayfish should have had a biological half-life for CH_3Hg of 24–53 days. Medium and large crayfish should have had biological half-lives of 63–77 and 87–116 days, respectively. These predicted half-lives are considerably less than the observed half-lives.

Fractional clearance rates of CH_3Hg determined for a number of fish species, such as northern pike (*Esox lucius*) (Järvenpää et al. 1970), rainbow trout (*Oncorhynchus mykiss*) (Ruohtula and Miettinen 1975; Rodgers and Beamish 1982), and plaice (*Pleuronectes platessa*) (Pentreath 1976), as well as the benthic invertebrate *Hyalella azteca* (Lloyd 1979), fit the above relationship established by Sharpe et al. (1977). In contrast, the clearance rate of CH_3Hg from *Daphnia magna* was much slower than predicted (Trudel 1980). The logarithm of CH_3Hg fractional clearance rates was plotted by Trudel (1980) for *Daphnia magna* (Trudel 1980), *Daphnia pulex* (Huckabee et al. 1975), *Hyalella azteca* (Lloyd 1979), and the marine benthic shrimp *Lysmata seticaudata* (Fowler et al. 1978) against the logarithm of wet masses of these organisms. The equation describing the relationship between fractional clearance rate and mass for Crustacea was as follows:

$$K_{cl} = 0.0006\ W^{-0.78}$$

This equation predicted fractional clearance rates for *O. virilis* that were closer to the values observed in this study. Small crayfish were predicted to have a biological half-life for CH_3Hg of 1155–3466 days. Medium and large crayfish were predicted to have biological half-lives for CH_3Hg of 4077–5776 and 6301–9902 days, respectively. If these predicted half-lives are similar to actual depuration rates by these organisms, a loss rate would be very difficult to detect over the lifetime of this crayfish species, let alone over 166 days. It is important to realize that the relationship between fractional clearance rate and body mass proposed by Trudel (1980) for crustaceans was derived from clearance data for very small organisms (a zooplankter, an amphipod, and a shrimp), with wet masses ranging from approximately 5×10^{-4} to 0.8 g. There is uncertainty associated with extrapolating from these data to crayfish, which initially weighed anywhere from 1 to 15 g.

In light of the fact that this crayfish species has an average life-span of 40 months (Momot 1967, 1978; Momot and Gowing 1977), we conclude that, over their life-span, *O. virilis* can lose at most only very small amounts of CH_3Hg. Because of the close association of crayfish with sediments, their significance as a trophic link in aquatic systems, and their importance as a food source, crayfish could be a significant vector for CH_3Hg transfer to consumers of these crustaceans. Even though humans do not commonly consume crayfish, they still consume sport fish, such as smallmouth bass (*Micropterus dolomieu*), which are themselves direct consumers of crayfish.

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Modelling the impact of disease-induced mortality in an exploited population: the outbreak of the fungal parasite *Ichthyophonus hoferi* in the North Sea herring (*Clupea harengus*)

K.R. Patterson

Abstract: A model of disease dynamics in an exploited stock was developed to assess the impact of the recent outbreak of *Ichthyophonus hoferi* in North Sea herring. Rates of infection by the disease and mortality rates of infected fish were estimated from field observations of prevalence, relative abundance, and commercial catches. At the height of the outbreak infection rates and mortality rates were about 0.4 year^{-1} and 1.5 year^{-1}, respectively. Parameterization of infection rate as a year-class and age effect was more complex and did not provide a better fit than a year and age parameterization. The impact of *I. hoferi* on the North Sea herring stock was estimated to be significant but not catastrophic. Had the outbreak not occurred (but with catches being the same), the stock size would have been some 21% higher in 1994. Had the outbreak not occurred (but with fishing mortality being the same), the stock would have been 11% higher in 1994 and the catches from 1991 to 1994 would have been 9.6% higher. The consequences of using a standard stock assessment model in which nonfishing mortality is assumed constant when this assumption is violated are also assessed. A simple model is used to indicate the importance of disease-induced mortality.

Résumé : Nous avons élaboré un nouveau modèle de la dynamique d'une maladie dans un stock exploité pour évaluer l'impact d'une poussée récente d'*Ichthyophonus hoferi* chez le hareng de la mer du Nord. Les taux d'infection par la maladie et les taux de mortalité des poissons atteints ont été calculés à partir d'observations de terrain sur la prévalence, de l'abondance relative et des captures commerciales. Au sommet de la poussée, les taux d'infection et de mortalité étaient d'environ 0,4 et 1,5 par an, respectivement. La paramétrisation des taux d'infection par l'effet sur la classe d'âge et sur l'âge était plus complexe et ne donnait pas un meilleur ajustement que la paramétrisation par l'âge et l'année. Nous avons jugé que l'impact d'*Ichthyophonus hoferi* sur le stock de hareng de la mer du Nord était notable mais non catastrophique. Si la maladie n'était pas survenue (mais les captures étant égales), la taille du stock aurait été supérieure de quelque 21% en 1994. Si la maladie n'était pas survenue (mais que la mortalité par pêche était restée la même), la taille du stock aurait été supérieure de 11% en 1994, et les captures de 1991 à 1994 auraient été plus élevées de 9,6%. Nous évaluons aussi les conséquences du recours à un modèle standard d'évaluation des stocks dans lequel la mortalité non due à la pêche est considérée comme constante quand cette hypothèse n'est pas respectée. Un modèle simple sert à indiquer l'importance de la mortalité induite par la maladie.
[Traduit par la Rédaction]

Introduction

In evaluating the dynamics of exploited fish populations, natural mortality is usually assumed to be constant and time-invariant (Megrey 1989; Hilborn and Walters 1992). However, this assumption may in certain cases be wholly wrong. This can occur where an outbreak of an infectious disease causes spectacular fish kills over a short time period. Some of the best documented of such events have been the outbreaks of the fungal parasite *Ichthyophonus hoferi* in the western North Atlantic (Sindermann 1956, 1958, 1965, 1979). Epizootics have occurred repeatedly in herring stocks in the Gulf of Maine and the Gulf of St. Lawrence, and have been characterized by large numbers of dead fish seen floating at the surface, lying on the bottom, washed up on beaches, or caught dead in demersal trawls (Cox 1916; Fish 1934; Templeman 1954; Tibbo and Graham 1963). Subsequent to these observations, catches of herring declined markedly despite a sustained effort in the fisheries. An example is given in Fig. 1*a*, showing a marked decline in catches after the outbreak. Sindermann and Chenoweth (1993) reviewed these events.

Short-term, parasite-induced population depletion events are not limited to herring stocks. Simons and Ayres (1991) reported that an exploited population of razor clams (*Siliqua patula*) in Washington state suffered mortalities of up to 95% owing to an unidentified gill parasite. An exploited stock of eels (*Anguilla anguilla*) in Lake Balaton, Hungary, suffered severe mortalities as a result of an outbreak of nematodes (*Anguillicola crassus*) (Biro 1992). Sea lampreys (*Petromyzon marinus*) cause significant mortalities in trout in the Great

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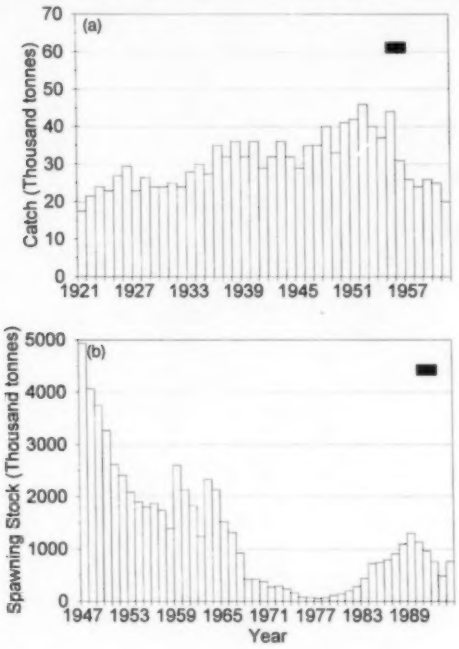
Fig. 1. *Ichthyophonus hoferi* outbreaks and herring stocks. The black bars indicate the approximate timing of *I. hoferi* outbreaks. (*a*) Landings of herring from the Gulf of St. Lawrence. Redrawn from Sindermann and Chenoweth (1993). Note the decline in landings after the outbreak (no estimates of stock size are available). (*b*) Estimates of stock size of North Sea herring, from Anonymous (1995). Note the decline in stock size after the outbreak, despite TAC-regulation intended to keep stock size stable.



Lakes (Spangler and Jacobson 1982). Furthermore, the highly valuable fishery for black abalone (*Haliotis cracherodii*) in California crashed despite the careful application of modern fishery management practices. A coccidian parasite was suspected of causing catastrophic mortality in the population (Davis 1993). The problem is therefore one of wide scope.

Despite the important effects that diseases may have on the abundance of exploited species, there has been relatively little attention paid to modelling such effects, and even where such studies have been attempted their purpose has usually been to draw general conclusions about the likely response of a system to different types of management action, rather than to estimate parameters and to make quantitative predictions. This has generally been due to the sparsity of the data compared with the complexity of the models. Most modelling attempts draw on the pioneering work by Anderson and May (1978, 1979), and Anderson (1982).

Theoretical aspects of stock management under conditions of density-dependent parasite mortality were examined by Dobson and May (1987), who based their study on the Schaefer (1954) biomass-dynamic model. From theoretical first principles, they were able to make general conclusions on the appropriate response of management to various conditions of disease-induced mortality. However, problems of parameter estimation were not addressed. Similarly, Des Clers and Wootten (1990) described a model with four ordinary differential equations defining the rates of transition between stages of the life cycle of the sealworm *Pseudoterranova decipiens*. The states of the model were the life stages of the parasite in seal, cod, and crustacean hosts as well as a free-living stage. To calculate predictions from this model it was necessary to specify values for 19 parameters, even though the model was not age structured and only an equilibrium solution was found. Most of the 19 parameters were poorly defined by data and were set to arbitrary values. Similarly, Kuris and Lafferty (1992) used a differential equation model of exploited crustacean populations subject to parasite-induced infertility to explore qualitatively the performance of different management strategies. Klinck et al. (1989), Hofmann et al. (1989), and Powell et al. (1994) did similar work in modelling the transmission of parasites and their effect on the growth of infected oysters. The usefulness of all these models lies, as in Dobson and May's work, in drawing general conclusions about the dynamics of the system rather than in estimating parameters and making specific predictions from a time series of observations.

Lorenzen et al. (1991), working on the dynamics of lymphocystis disease in estuarine flounder (*Platichthys flesus*), described a model with only 11 parameters, with which by assuming equilibrium they were able to predict infection and immunity profiles with respect to length and age. The model represented fish in four states (susceptible, immune, infected, infectious) and their transition between these states. These predicted profiles compared very well with the observed data. This, together with the work of Dwyer and Elkington (1993) on gypsy moths, is one of the first works in this area to take a conventional modelling approach by comparing model predictions with observed data. However, the approach of Lorenzen et al. (1991) also relied on the assumption of equilibrium conditions and density-dependent infection.

Here a modelling approach new to the field is used to analyse the dynamics of a stock undergoing an outbreak of a lethal disease. New models of stock and disease dynamics are formulated, and sufficient information is available to allow these models to be fitted to field observations in a conventional way, rather than making arbitrary, "reasonable" assumptions or inferring parameters from laboratory experiments. Equilibrium assumptions are not made. Instead the models are fitted to a time series of observations. This approach requires simplifying assumptions about disease dynamics, but even so the models are highly demanding of data. Information from a long time series of international surveys is used. The methods are applied to the exploited stock of herring, *Clupea harengus*, in the North Sea.

The North Sea herring *Ichthyophonus* outbreak

The North Sea herring has been of great economic, political, and even military importance in European waters since the first century AD (Parrish and Saville 1967). Some essential features of the stock are outlined below. There have been historical changes in migration patterns that are probably related to hydrographic changes (Corten and van de Kamp 1992), but in recent years the dominant pattern (Fig. 2) has been that adult herring spawn between August and December along the eastern coast of the United Kingdom, then migrate northeastwards and spend the winter in the cold waters of the Norwegian deeps. In the spring, the fish spread out over the northern and

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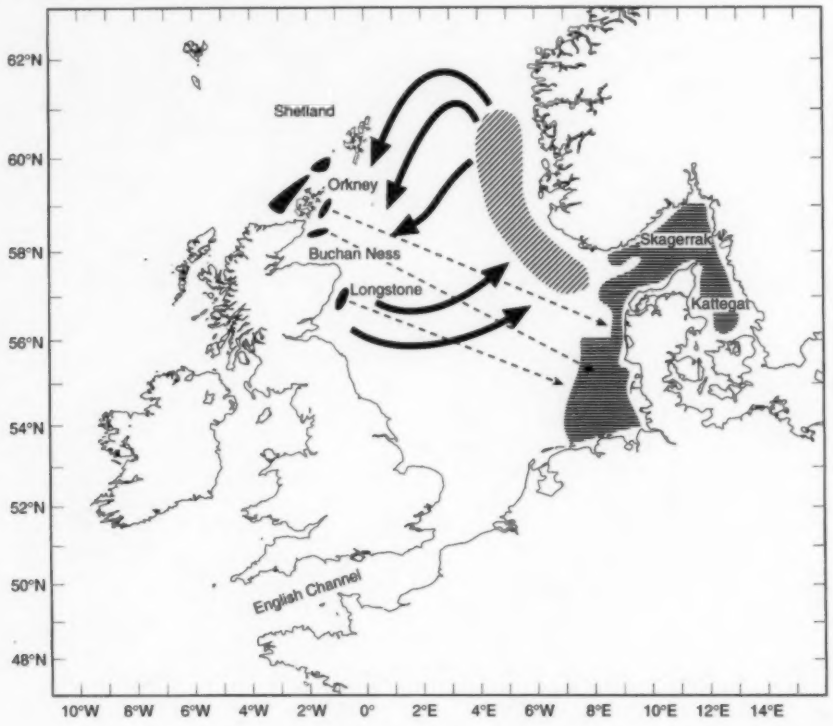
Fig. 2. Principal migrations of the Buchan component of the North Sea herring stock. In recent years this has been the dominant component. Diagonal shading, overwintering area of the adult stock; solid areas, spawning grounds; bold arrows, annual migrations of the adult stock; horizontal shading, nursery areas; broken arrows, advection of larvae from spawning grounds to the nursery areas. The juvenile fish leave the nursery areas and join the adult stock at about 2 years of age.



central areas of the North Sea to feed during the plankton blooms, and by midsummer they start to form dense shoals that move towards the spawning grounds. The eggs are laid demersally, but on hatching the larvae drift eastwards, eventually metamorphosing and passing their juvenile phase of life in the shallow, inshore waters of the German Bight and the Skagerrak and Kattegat areas. This stage lasts about 2 years, after which the fish join the main adult stocks in the central and western North Sea. Adult fish are exploited mostly during the prespawning aggregations, and there is also an inshore fishery for juvenile fish. The stock supported catches between 300 000 and 600 000 t in most years of peace since 1900, but the stock underwent a collapse in the late 1970s, which is usually attributed to a combination of a period of high catches in the 1960s (up to 1 200 000 t in 1964; Anonymous 1995) and environmentally driven poor recruitment (Corten 1986). Estimates of stock size (Anonymous 1995; made using models that take no account of disease-induced mortalities) show a decline from 5 000 000 t in 1947 to 50 000 t in 1977, with a subsequent rebuilding of the stock to 1 300 000 t in 1989. Since 1990 the stock has again declined, concomitant with reports of an *Ichthyophonus* outbreak in 1991 (Anonymous 1993), and current estimates of stock size in 1994 are around 760 000 t (Fig. 1*b*).

The organism that caused this outbreak is *Ichthyophonus hoferi*, a phycomycete fungus with a low host specificity affecting mainly marine fish worldwide. It occurs commonly in haddock (*Melanogrammus aeglefinus*), plaice (*Pleuronectes platessa*), and herring, but there are also records from salmonids and even amphibians and crustaceans (McVicar 1984). Symptoms include nervous disorders, abdominal distension resulting from kidney failure or swelling of visceral organs, and wasting of musculature. White nodules and streaks up to several millimetres long can spread through the musculature and body organs. In its acute form, tissues of vital organs such as the heart and brain are replaced with fungal hyphae and necrotic and granulomatous reactions (Rahimian 1994). Haddock appear to tolerate sustained infection, but in herring the disease is thought to be lethal. Laboratory experiments by Sindermann (1958) and McVicar (1981) suggest that fish with externally visible signs of infection can be expected to survive for around 3 months. Transmission of the disease is thought to be by oral ingestion of spores from the water, or by feeding on infected fish. This latter transmission route has implications for aquaculture when feeds may be made from infected fish. A mobile amoeboid stage of the disease also exists. In the Gulf of St. Lawrence epizootics up to 25% infection was recorded, and during an epizootic of plaice in the North Sea, infection rates of up to 10% with concomitant mortalities were observed.

The problem of assessing the impact of the disease outbreak has been addressed in five stages. These have been intended to follow a logical sequence.

(*i*) The hypothesis that natural mortality is higher for those

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Table 1. Estimates of the proportion of *I. hoferi* infection (percentage) from international acoustic surveys and from Norwegian summer and autumn surveys.

Year	Age (rings) 2	3	4	5	6	7	8	9+	Sample size
International acoustic surveys									
1992	0.0	0.72	4.96	12.28	14.09	0.0	11.29	0.0	5094
1993	0.1	0.6	3.4	8.3	5.0	3.0	4.8	6.9	6550
1994	0.11	0.20	1.81	0.0	2.81	0.76	3.48	3.05	2335
Norwegian summer acoustic surveys									
1991	1.4	3.4	11.6	15.1	18.0	15.0	0.0	0.0	537
1992	1.0	1.6	0.9	7.6	11.5	15.3	9.4	1.9	773
1993	0.1	0.0	1.3	0.4	3.9	6.3	16.5	21.1	1258
1994	0.0	0.0	1.0	0.0	0.3	0.9	3.4	14.3	1151
Norwegian autumn trawl surveys									
1991	0.0	1.7	10.6	1.7	7.2	0.9	0.0	0.0	196
1992	0.0	3.4	5.0	4.3	5.7	3.4	1.1	27.0	805
1993	0.9	0.0	0.4	0.9	0.9	0.69	0.0	3.4	625
1994	0.6	0.2	0.6	0.0	1.7	1.2	0.0	0.0	589

Note: Fish aged 0 and 1 are excluded. Data are from Anonymous (1995) and from E.J. Simmonds (personal communication, Marine Laboratory, Aberdeen, Scotland, U.K.).

Fig. 3. Example of sampling and prevalence rates for *I. hoferi* in the 1992 international acoustic survey. Numbers in each rectangle are the number of infected fish/number of fish examined. These statistics are summarized in four principal survey areas and an overall percentage prevalence was calculated. Rectangles with no numbers were not sampled. Prevalence in the population was calculated from the area-based infection rates, raised according to the abundance of herring in each rectangle. A simple linear interpolation procedure was used to fill in unsampled rectangles. Modified from Simmonds et al. (1993).

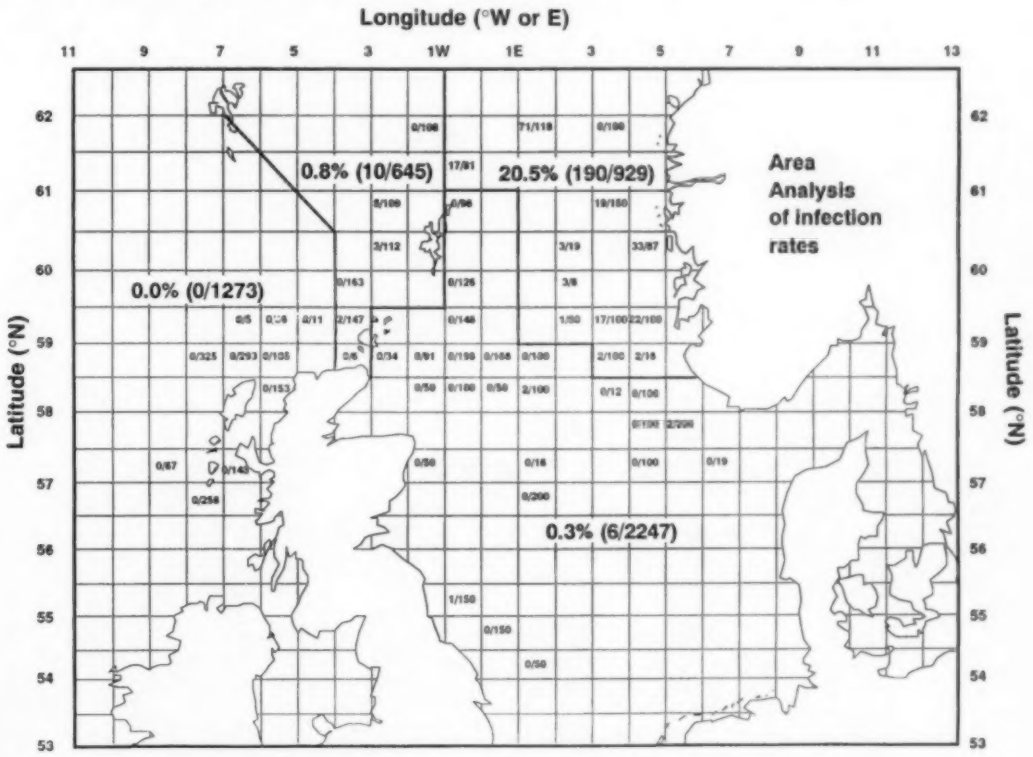

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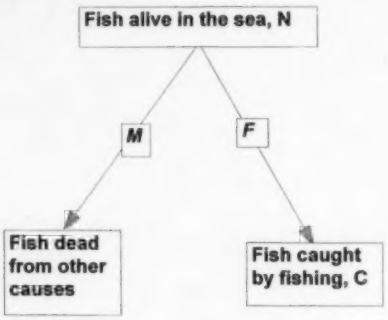
Fig. 4. States and rates of change between states assumed in the conventional stock assessment model. N, population abundance; M, natural (nonfishing) mortality; F, mortality resulting from fishing; C, numbers of fish caught.



ages and in those years when high levels of disease infection were observed is examined using a simple extension to the conventional model, here referred to as the simple model. This stage is necessary to ensure that unnecessary complexity is not being introduced.

(*ii*) A new structural model is formulated to represent the outbreak of the disease in the stock. This model serves three purposes: it can be used to generate simulated data from arbitrary prior assumptions, to estimate parameters of stock abundance, fishing mortality, infection rate, and disease-induced mortality, and thirdly to explore alternative scenarios in projections. This model is referred to as the extended disease dynamics model.

(*iii*) Characteristics of the conventional stock assessment are examined for features indicating that the conventional model is inappropriate. A simulation approach is used to generate a pseudo-data set representing a stock undergoing an outbreak of a lethal disease, and the conventional assessment model is fitted to this data set. Trends in residuals, stock size, and fishing mortality estimates from this simulation experiment are compared with those seen in the conventional assessment.

(*iv*) A nonlinear least-squares method is used to estimate the parameters of the extended disease dynamics model. Two alternative formulations are fitted and compared.

(*v*) The parameter estimates are used to explore alternative outcomes for the development of the stock, under conditions that the disease outbreak had not occurred. This allows an assessment of the impact of the outbreak on the stock and its effect on the yield to the fishery.

Methods

Available data

Research vessel surveys of herring abundance have been carried out in various forms since 1972. These have included surveys of larval abundance (Heath 1993), acoustic surveys (e.g., Simmonds et al. 1993), and demersal trawl surveys (Anonymous 1994*b*). These data, together with estimates of catches in number at age over the period 1975–1994, are available in Anonymous (1995) and have been used for the present analysis (also available on request from the author).

In addition to the routine surveys and when the *I. hoferi* outbreak became obvious, it was decided to introduce routine sampling of the catches made during the acoustic surveys to record the numbers of infected and uninfected herring. The criterion used for this examination was macroscopic infection of the heart for the white nodules that are characteristic of this disease (Anonymous 1993).

Available information on the proportion of herring infected with the disease from Norwegian summer acoustic surveys, Norwegian autumn trawl surveys, and international summer acoustic surveys is summarized in Table 1. In 1991 only Norway recorded parasite information, hence although Norwegian surveys form a component of the international acoustic surveys, it is necessary to present and to use this information separately. Rates of infection in the whole stock were calculated using abundance-weighted rates of infection in the samples. The number of fish examined for infection by survey rectangle (1° latitude × 0.5° latitude) is shown in Fig. 3 to illustrate the level of spatial coverage achieved in the international acoustic surveys in 1992. Similar coverage was achieved in the other years.

Methods used to assess herring stocks

To assess the usefulness of the disease dynamics model it proved useful to define a conventional assessment model to be used as a reference when extending the principles to include disease-induced mortality. The model is essentially that used by Anonymous (1995), who also reviewed the appropriateness of the assumptions that are incorporated. It is an integrated statistical catch at age model including a separability constraint (Deriso et al. 1985; Kimura 1986; Gudmundsson 1986).This is a three-state model (Fig. 4) in which fish either (1) are alive in the sea, (2) are caught by fishermen, or (3) die from causes other than fishing. Fish move only between states 1 and 2 and between states 1 and 3, or else remain in state 1. The number of uninfected fish in the sea is represented with N, fishing mortality with F, natural mortality with M, the number of fish caught with C, and the number of fish dying (including those caught) in period of duration t starting at time 0 with D. Three simple equations model the transition rates between states:

$$(1)\qquad \frac{dC}{dT} = FN; \quad \frac{dD}{dt} = -(F+M)N; \quad \frac{dN}{dt} = (F+M)N$$

and from these,

$$(2)\qquad N_t = N_0 - \int_{i=0}^{i=t} N_i(F+M)\,di$$

$$C_t = \int_{i=0}^{i=t} N_i F\,di$$

Conventionally, catch and population are analysed by year and age (so $t=1$ year and units of F and M are year^{-1}). Fishing mortality is assumed to be separable into a component that changes between years y (f_y) and one that changes between ages a (S_a):

$$(3)\qquad F_{a,y} = f_y S_a$$

Available information consists of catches $C_{a,y}$ and of one or more survey indices j of stock size $U_{a,y,j}$. From eqs. 1–3, predicted catch $\hat{C}$, population abundance N, fishing mortality F, and natural mortality M are assumed to be related according to the catch equation and observed with log-normal error:

$$(4)\qquad \ln(\hat{C}_{a,y}) = \ln\left(\frac{F_{a,y}}{F_{a,y}+M_{a,y}} N_{a,y}(1-\exp(-F_{a,y}-M_{a,y}))\right) + \varepsilon^c_{a,y}$$

where $t=1$ year and is omitted. ε^c is distributed as $N(0, \sigma^2_c)$. Survey indices of abundance (U) are assumed to be related to stock size by a simple proportionality coefficient, termed a catchability, Q, and observed with log-normal error:

$$(5)\qquad \ln(U_{a,y,j}) = \ln(Q_{a,j} N_{a,y}) + \varepsilon^u_{a,y}$$

or, if the survey index Ub measures spawning stock biomass SSB,

$$(6)\qquad \ln(\mathrm{Ub}_y) = \ln(\mathrm{Qb}\,\hat{\mathrm{SSB}}_y) + \varepsilon^b_y$$

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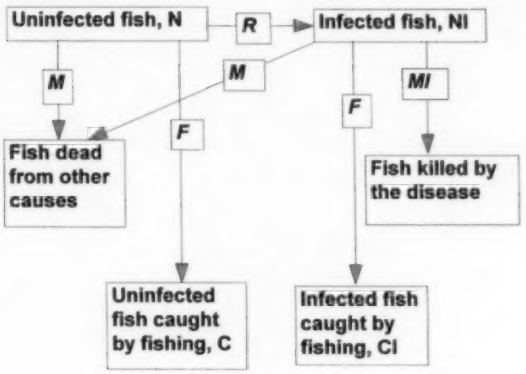
Fig. 5. States and rates of change in the extended model. *N*, population abundance; *M*, natural mortality from causes other than parasite mortality; *F*, mortality resulting from fishing; *C*, numbers of uninfected fish caught; CI, numbers of infected fish caught; NI, abundance of infected fish; MI, rate at which infected fish die from the disease.



in which

$$(7)\qquad \hat{SSB}_y = \sum_a N_{a,y}\, W_{a,y}\, O_{a,y}\, (\exp(-F_y\, S_a\, PF - M_{a,y}\, PM))$$

where PF and PM are the proportions of fishing and of natural mortality that occur prior to spawning in each year, $O_{a,y}$ are estimates of the proportions of fish of age a in year y that spawn, and $W_{a,y}$ are estimates of mass at age and year. Errors ε^u and ε^b are assumed to be distributed as $N(0,\sigma_u^2)$ and as $N(0,\sigma_b^2)$, respectively.

Population sizes are estimated from a nonlinear least-squares fit of predicted log catch ($\hat{C}_{a,y}$) and j predicted survey indices ($U_{a,y,j}$, Ub_y) to observed values:

$$(8)\qquad \sum_{a,y} (\ln(C_{a,y}) - \ln(\hat{C}_{a,y})^2 + \sum_{a,y,j} (\ln(U_{a,y,j} - \ln(Q_{a,j}\, N^*_{a,y})^2 + \sum_y (\ln(Ub_y) - \ln(Qb\, \hat{SSB}))^2$$

$N^*_{a,y}$ are population sizes in year y and at age a adjusted for the date of the survey within year y. Parameters to be estimated are the population sizes N at the last year and age, survey catchabilities $Q_{a,j}$, fishing mortalities f_y, and age-specific selection S_a.

For reference purposes an assessment model was fitted to catch at age and survey observations by minimizing the term in eq. 8. This fit differs only in minor ways from the assessment model used in Anonymous (1995) and is here termed the base-line fit as it is used as the reference fit with which the parasite dynamic models are compared. A conventional nonlinear least-squares algorithm using a modification of Newton's method was used for this and subsequent model fits. The variance–covariance matrix was estimated by $2\,SSQ(\mathbf{J}^T\mathbf{J})^{-1}/(df)$, where SSQ is the sum of squared residuals, df the degrees of freedom, and **J** the Jacobian at the solution. NAG routines E04FDF and E04YCF were used (Numerical Algorithms Group, Oxford). All parameters were log-transformed for the fitting procedure, hence the confidence intervals are asymmetric.

A simple model

Perhaps the simplest assumption that can be made about the effect that the disease might have on the population is that when observed prevalences are high, natural mortality increases. Let MO represent the natural mortality excluding disease-induced mortality, NI represent the numbers of infected fish, and N represent the numbers of uninfected fish. Assuming a linear relationship for simplicity,

Table 2. Parameter estimates used to create the simulated data set.

Year-specific parameters			
Year (y)	Recruitment ($N_{0,y}$)	Fishing mortality (F_y)	Year-specific infection rate (R_y)
1985	1 000 000	0.4	0.0
1986	1 000 000	0.4	0.0
1987	1 000 000	0.4	0.0
1988	1 000 000	0.4	0.0
1989	1 000 000	0.4	0.0
1990	1 000 000	0.4	0.0
1991	1 000 000	0.4	0.6
1992	1 000 000	0.4	0.5
1993	1 000 000	0.4	0.2
1994	1 000 000	0.4	0.01

Age-specific parameters			
Age (a)	Selection (S_a)	Age-specific infection rate (R_a)	Natural mortality (aM)
0	0.025	0	1.0
1	0.25	0	1.0
2	0.5	0.2	0.3
3	1.0	1.0	0.2
4	1.0	1.0	0.1
5	1.0	1.0	0.1
6	1.0	1.0	0.1
7	1.0	1.0	0.1
8	1.0	1.0	0.1

Note: Bounds of the simulated data: year range, 1985 to 1994; age range, 0 to 8; surveys, 1 survey covering ages 2–8 and years 1985–1994. Recruitment (population abundance at age 0) = 1 000 000 in all years. Fully recruited fishing mortality (F_y) = 0.4 in all years. MI, the additional mortality rate of infected fish, was assumed to be constant at 1.565 year^{-1}.

$$(9)\qquad M_{a,y} = MO_a + k\,\frac{NI_{a,y}}{N_{a,y} + NI_{a,y}}$$

This is analogous to the assumption made about density dependence in natural mortality by Haist et al. (1993). A simple way to estimate the ratio $NI/(N + NI)$ is to make the assumption that the records of *I. hoferi* infection in the surveys (Table 1) are observed without error; they become constants to be included in the model. The significance of the inclusion of this variable natural mortality term in the model can be assessed by the conventional extra sum of squares principle (Draper and Smith 1981) in comparison with the base-line model fitted with a fixed natural mortality. The goodness of fit of the three series of prevalence data (international acoustic, Norwegian summer, and Norwegian autumn) can be assessed separately. The treatment of estimates of the proportions of infected fish from surveys as exact is far from ideal, but it is useful for indicative purposes. Parameters for this simple model were estimated by including the term in eq. 9 in the structural model for the base-line assessment, minimising the term in eq. 8 with a single additional parameter K.

Extended disease dynamics model

To develop a model that can be used to predict infection rates it becomes useful to replace the conventional three-state model of stock dynamics (Fig. 4) with a model including an additional, infected state (Fig. 5). To the traditional states N and C (fish in the sea and fish caught) the new state NI (infected fish) is added, which is subject to both fishing and to natural mortality (which excludes mortality from the disease). Fish are infected (pass between states N and NI) at a rate R. A further state for catches of infected fish (CI) can be added.

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Table 3. Comparison of model fits with and without the variable-natural mortality term, based on either the international acoustic, Norwegian summer, or Norwegian autumn survey estimates of parasite prevalence.

Model	Sum of squared residuals	Degrees of freedom	F (inclusion)	$P(F)$	Estimate of K[a]
Base line	11.1976	159			
International acoustic	10.8760	158	4.566	< 0.05 (1, 159 df)	2.84 (1.15–7.01)
Norwegian summer	11.1961	158	0.0021	~1.0 (1, 159 df)	2.34 (1.06–5.14)
Norwegian autumn	10.9518	158	3.4904	$0.1 > P > 0.05$	4.481 (1.86–10.78)

[a]Values in parentheses are 95% confidence limits.

Infected fish are assumed to be subject to an additional mortality rate MI, the rate at which infected fish die from the disease. This rate will be referred to herein as disease-induced mortality. The term natural mortality (M) will be used to describe mortality from all causes except for fishing and mortality resulting from the disease. Infected fish are here defined as those fish exhibiting detectable symptoms of parasite infection, specifically, those fish that could be identified from macroscopic examination during the surveys as having the disease. It is assumed that fish exhibiting no symptoms are not subject to disease-induced mortality.

The stock dynamics are described by the following linked ordinary differential equations:

$$(10)\quad \frac{dN}{dt} = -(F + M + R)N$$

$$(11)\quad \frac{dNI}{dt} = RN - (M + MI)NI$$

$$(12)\quad \frac{dC}{dt} = FN$$

$$(13)\quad \frac{dCI}{dt} = F\,NI$$

By integration from time $i = 0$ to time $i = t$ (where t will conventionally be 1 year), the development of the infection in the population can be modelled. The uninfected population size at time t is given by

$$(14)\quad N_t = N_0 - \int_{i=0}^{i=t} (F + M + R)N_i$$

$$= N_0 \exp(-t(F + M + R))$$

and the number of infected fish at time t is given by

$$(15)\quad NI_t = NI_0 + \int_{i=0}^{i=t} -NI_i(F + M + MI) + N_iR$$

$$= \left(NI_0 + \frac{NR}{(MI - R)} \exp(t(MI - R) - 1\right) \exp(-t(F + M + MI))$$

The number of uninfected fish caught between times 0 and t is

$$(16)\quad C_t = \int_{i=0}^{i=t} FN_i$$

$$= \frac{F}{F + M + R}(1 - \exp(-t(F + M + R))$$

and the number of infected fish caught in the same time period is

$$(17)\quad CI_t = \int_{i=0}^{i=t} F\,NI_i$$

$$= \frac{F \exp(-t(MI + F + M))(NI_0MI - R(NI_0) + N_0)}{(MI + F + M)(MI - R)}$$

$$- \frac{FN_0R \exp(-t(F + M + R))}{(MI - R)(F + M + R)} + \frac{F(F\,NI_0 + NI_0(MI + R) + N_0R)}{(MI + F + M)(F + M + R)}$$

Equations 14–17 can be used in place of the conventional catch equations in the base-line stock assessment structural model, so that the integrations between time 0 and time t become integrations between year y, age a, and year $y + 1$, age $a + 1$.

There are 28 year- and age-specific infection rate parameters that could be estimated, but only 49 survey observations of prevalence of the disease. The model is likely to be overparameterized unless some constraints are imposed. As such constraints are necessarily arbitrary, the following two hypotheses for constraining the analysis were evaluated for comparative purposes.

(*i*) Infection rate R is assumed to be age dependent and also year dependent, but there is no interaction between the two effects. This allows a reparameterization of R as

$$(18)\quad R_{a,y} = RA_aRY_y$$

where RA is an age-specific infection rate and RY is a year-specific infection rate. Alternatively,

(*ii*) Infection rate R is assumed to be dependent on year-class and on age. Under this hypothesis the model may be parameterized as

$$(19)\quad R_{a,y} = RC_cRA_a$$

where RC represents the rate of infection for year-class C.

Simulations using the disease dynamics model

A simulated data set loosely based on the main features of the North Sea herring stock was generated using eqs. 14–17 and parameter values given in Table 2. This represents a stock with constant recruitment and constant fishing mortality over the years 1985–1994, which is subjected to an outbreak of a disease that begins in 1991, decreases slightly in 1992, decreases to a lower level in 1993, and has almost disappeared in 1994. Vulnerability to the disease starts at age 2 and full susceptibility to the infection is assumed to start at age 3 and remain constant at older ages. Predicted catches from the stock were calculated by summing expected infected and uninfected catches (eqs. 16 and 17). It is also assumed that a single age-structured survey of the stock is made using a method that measures the abundance of both infected and uninfected fish aged 2–8. This corresponds roughly to the acoustic survey used (among other sources of information) to measure stock size.

This simulated data set was then analyzed using a stock assessment procedure similar to that used to assess the North Sea herring stock. The structural relationships given in eqs. 2–6 were assumed to hold, and parameters were estimated by a nonlinear minimization of the term (eq. 8) above. The fishing mortality and population size estimated by this procedure were compared with those used to construct the simulated data, and also with the parameter estimates obtained in the base-line assessment.

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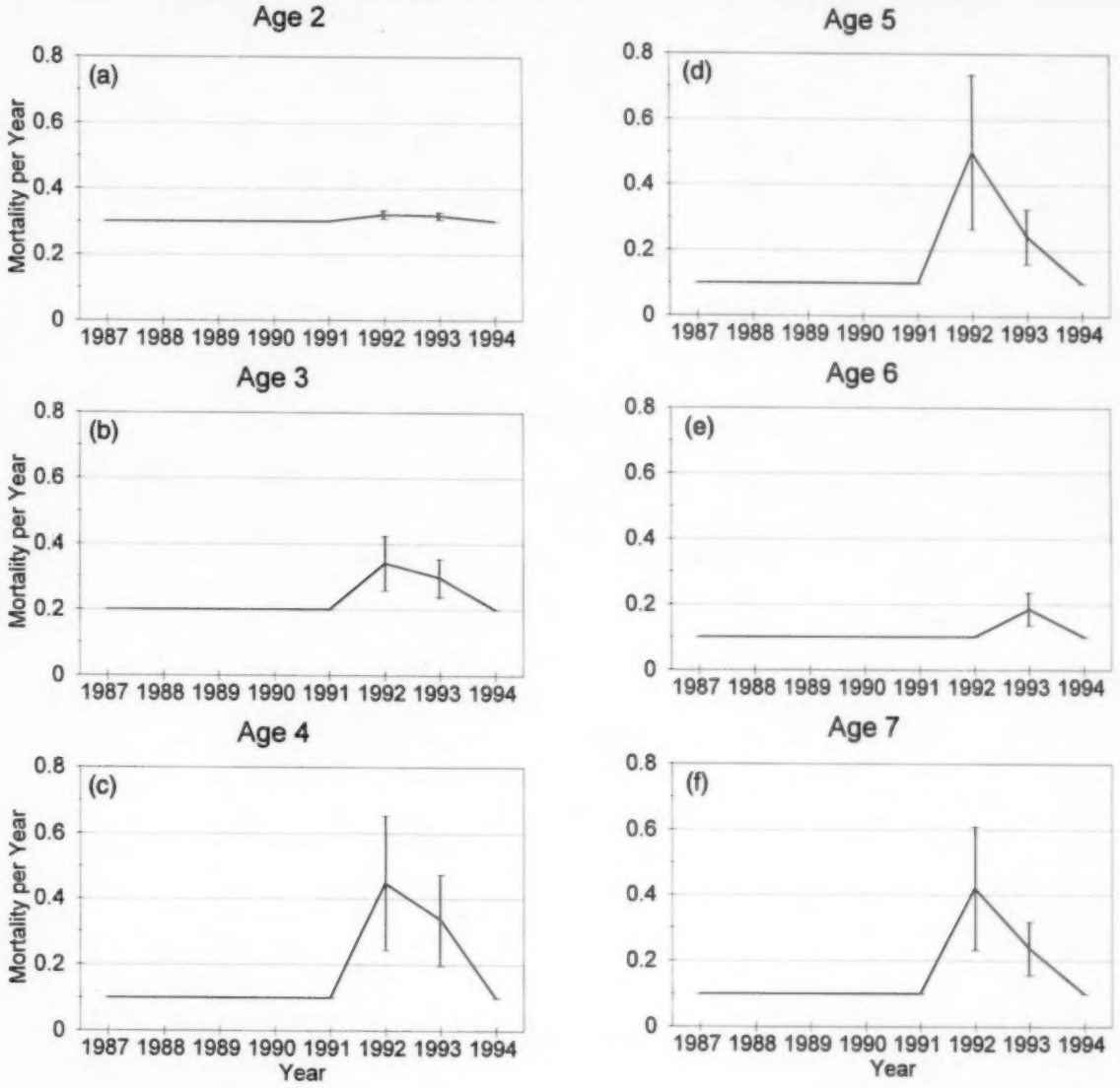
Fig. 6. Nonfishing mortality estimated using the simple model, by age (in winter rings). Error bars show ±95% confidence limits.



Estimating parameters of the disease dynamics model

The development of the disease in the population can be analysed by replacing the conventional structural model (eqs. 2–7) with the disease dynamics structural model (eqs. 14–17). As a simple approximation, consider the $P_{a,y,j}$ observations of the prevalence of the disease at age a in year y in survey j as independent, normally distributed observations with variance $V_{a,y,j}$. Assume that in the jth survey a sample of size $L_{a,y,j}$ is taken from a fish population of age a in year y containing NI infected fish and N uninfected fish. If the sample were drawn randomly from a uniform population, then the expected proportion of infected fish (prevalence, $\hat{P}_{a,y,j}$) would be binomially distributed. Writing $\hat{P}_{a,y,j} = \mathrm{NI}_{a,y}/(\mathrm{NI}_{a,y} + N_{a,y})$, the variance of the observed proportion $P_{a,y,j}$ would be $V_{a,y,j} = \hat{P}_{a,y,j}(1 - \hat{P}_{a,y,j})/L_{a,y,j}$. As $\hat{P}_{a,y,j}$ tends to zero, then the variance of $P_{a,y,j}$ tends to zero also. Following Haist et al. (1993) and to render the model less sensitive to the occurrence of unlikely events, the variance estimate is calculated as $V_{a,y,j} = (\hat{P}_{a,y,j}(1 - \hat{P}_{a,y,j}) + 0.01)/L_{a,y,j}$. These observations can then be included in the objective function as an additional term:

$$(20)\quad \sum_{a,y,j} \frac{(P_{a,y} - \hat{P}_{a,y,j})^2}{V_{a,y,j}}$$

The $\hat{P}$ values are calculated from N and NI in the structural model from eqs. 14 and 15. Observations of $P_{a,y,j}$ from the international acoustic survey and from the Norwegian autumn survey have been used, but Norwegian summer survey observations have been excluded as they are included in the international acoustic survey, and they did not improve the fit when included in the simple model. The numbers of fish sampled at each age and year were available from Norwegian survey reports (D. Skagen, quoted in Anonymous 1995), but only the age-aggregated totals sampled for *I. hoferi* per year were

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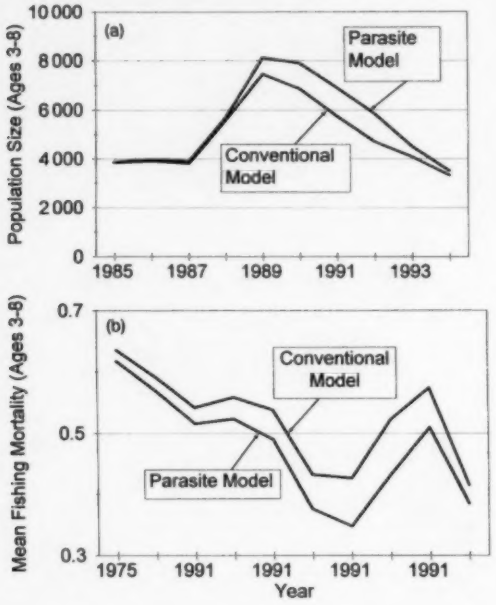
Fig. 7. Estimates of (*a*) stock abundance and (*b*) fishing mortality from the base-line assessment using the conventional model and from the simple parasite model.



reported for the international acoustic surveys. On the assumption of random sampling, an assumed sample size was calculated by allocating the sample totals across ages in the same proportion as the estimated age structure of the stock. However, in neither case was account taken of the spatial structure of the sampling.

A scaling problem exists when including this term in the objective function. In the base-line stock assessment and following the procedure of the International Council for the Exploration of the Sea (ICES) (Anonymous 1995), the variances of the survey and catch at age observations are not known, and rather than using an iterative reweighting procedure with attendant risks of numerical instability, the variance estimates are replaced with arbitrary weights. Expression (20) approximates a variate with zero mean and unit variance; it is desirable to include a scaling factor so that the parasite prevalence terms are given equal weight to the other observations in the model fit, which can be achieved by weighting the parasite prevalence observations by the variance of the other observations. From the base-line model fit, the weighted sum of squared residuals was 11.19 for 159 df, hence the term (20) was included in the objective function and weighted by 0.0704. In a number of trial runs, the parameter estimates obtained were found to be highly robust to changing the value of this weighting factor.

Throughout, the following simplifying assumptions have been made. (*i*) The surveys return unbiased estimates of the proportions of infected and uninfected fish. (*ii*) There is no substitution of natural mortality (not caused by disease or fishing) between uninfected and infected fish, i.e., M on uninfected fish = M on infected fish. (*iii*) Fish are either infected or uninfected, with no appreciable time lag in transition between the states. Fish in the infected state are subject to an additional mortality rate MI, which does not depend on how long the fish have been infected. The infected state is the state in which macroscopic examination of the heart reveals the presence of the infection (this is the criterion used in field examinations). (*iv*) Fish in the infected state make no contribution to spawn deposition. Given the catastrophic systemic effects of *I. hoferi* infection (Anonymous 1993), this appears likely. (*v*) Populations are assumed to be homogeneous and mixed: spatial structure is ignored. Although spatial structure certainly exists and may well be important, there are too few observations to allow subdivision of the model into spatial components. (*vi*) Fishing mortality F and infection rate R are constant within each year and change abruptly on 1 January. (*vii*) Infected and uninfected fish are equally vulnerable to fishing mortality.

The model provides for observations of catches of infected fish to be included separately from those of uninfected fish. However, reliable estimates were not available and hence the predicted catches were calculated by summing the C and CI terms for each age and year. The full objective function was therefore

$$(21)\quad \sum_{a,y} (\ln(C_{a,y}) - \ln(\hat{C}_{a,y} + \hat{\mathrm{CI}}_{a,y})^2 + \sum_{a,y,j} (\ln(U_{a,y,j} - \ln(Q_{a,j}(N^*_{a,y} + \mathrm{NI}^*_{a,y})))^2 + \sum_{y} (\ln(\mathrm{Ub}_y) - \ln(\mathrm{Qb}\ \hat{\mathrm{SSB}}_y))^2 + \sum_{a,y,j} \left(\frac{\kappa}{V_{a,y,j}} (P_{a,y,j} - \mathrm{NI}^*_{a,y} / (N^*_{a,y} + \mathrm{NI}^*_{a,y}) \right)^2$$

where κ is the scaling factor for the prevalence observations, equal to 0.0704.

The parameters estimated in the nonlinear search were $F_{[1991-1994]}$, S_{0-7} (except S_4 and S_8 constrained = 1); RA_{2-7} (RA_0, RA_1 constrained = 0, and RA_8 constrained = 1); $\mathrm{RY}_{1991-1994}$, $N_{1991-1994,8}$; and $N_{1994,0-7}$. The outbreak was assumed to start in 1991, so all NI were constrained = 0 on 1 January 1991. Similarly, recruiting fish were assumed to be free of infection, so that $\mathrm{NI}_{1991-1994,0} = 0$. Other parameters were estimated within the structural model. The procedure was repeated with the same input data but with the parameterization of R given in eq. 21, parameters RC_c and RA_a being estimated instead of the year and age effects. N^* and NI^* are the uninfected and infected populations calculated for the date in the year when the surveys were made, upon prior specification of the proportions of F, M, and R in each year applicable before each survey is made.

Results and discussion

The base-line assessment

The fitted population parameters obtained using the base-line model were generally similar to those obtained in the ICES assessment (here, mean F on ages 2–6 in 1994 = 0.4163 compared with 0.5265; SSB in 1994 = 944 850 t compared with 792 094 t). The difference is due to the inclusion of the 1987 and 1988 acoustic surveys; the matter is discussed in detail in Anonymous (1995). In the following, disease dynamics models are compared with the base-line model fit calculated here.

Fitting the simple model

The model was fitted to catch, age composition, and survey information from Anonymous (1995) and parasite infection information in Table 1, using either the international acoustic, Norwegian summer, or Norwegian autumn surveys. A comparison of the sums of squares of the fits with that of the base-line assessment is given in Table 3. On the basis of the F tests, inclusion of the Norwegian summer acoustic survey estimates of prevalence was rejected ($p \sim 1.0$) and inclusion of the international acoustic survey estimates was accepted ($p < 0.05$). The case of the Norwegian autumn trawl survey is less clear-cut ($0.1 > p > 0.05$); however, it appeared reasonable to retain the observations within the model as it is not likely that the survey does provide estimates of prevalence of *I. hoferi* in the

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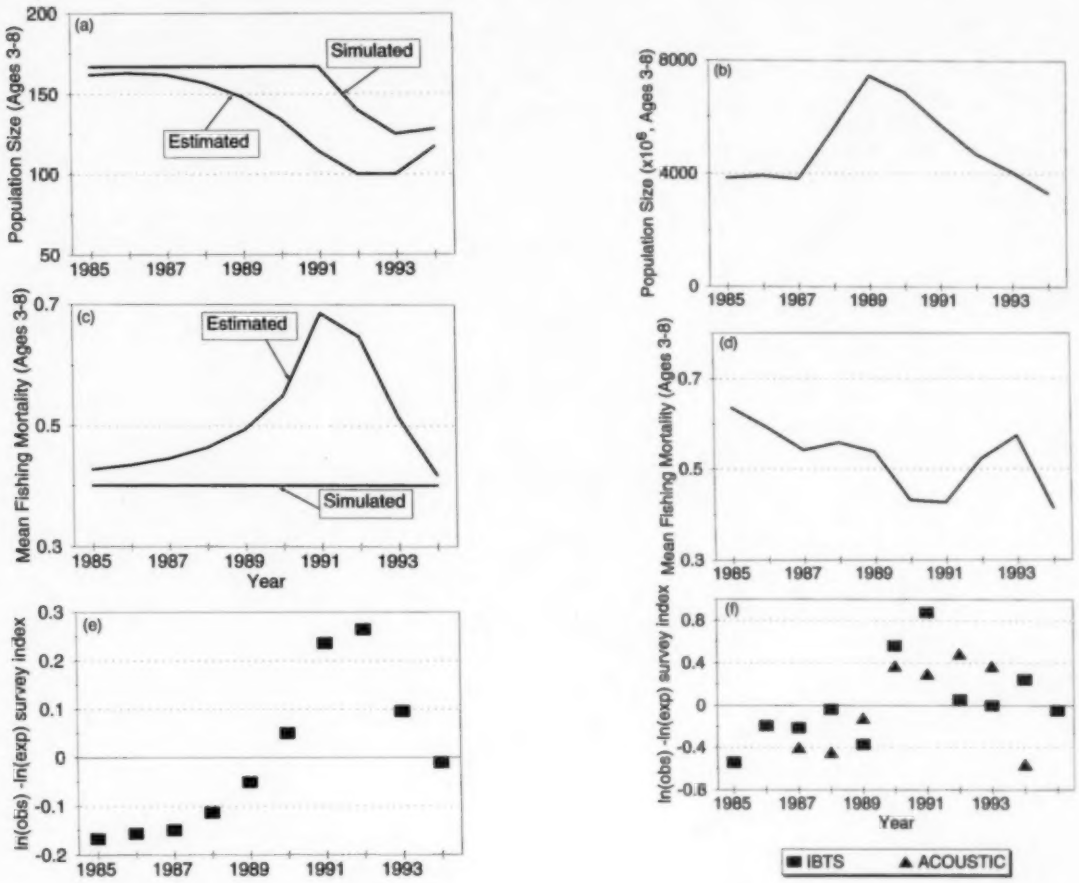
Fig. 8. (*a*, *c*, and *e*) Results and diagnostics from using a conventional stock assessment model to estimate population parameters from a simulated data set generated using the model given in eqs. 15–19 and the starting parameters given in Table 3. Estimates of stock size and fishing mortality are given together with the true values used to generate the data. (*b*, *d*, and *f*) Results and dignostics from the stock assessment of North Sea herring from Anonymous (1995). (*a–b*) Abundance of populations aged 3–8 years. (*c–d*) Mean fishing mortality of fish aged 3–8 years. (*e–f*) Log survey residuals at age 4.



population. Mortality estimates derived from the model fit are given in Fig. 6 by age, and a comparison of mortality and stock size estimates of the simple parasite model and the base-line model is given in Fig. 7.

The simple model fit showed that the international survey estimates of prevalence agreed well with the hypothesis that increasing prevalence indicates increased nonfishing mortality, and a reasonable fit was obtained to the Norwegian autumn surveys. However, the fit to the observations made in the Norwegian summer surveys alone was poor. This is not entirely surprising as these surveys cover the eastern half of the North Sea in midsummer, at which time the largest concentrations of the adult stock occur in the western half of the North Sea. In addition, the focus of concentration of the infection appears to have occurred in the extreme northwest of the distribution of this stock (Simmonds et al. 1994). The Norwegian surveys did not therefore cover the main centres of abundance nor of infection, and hence the poor fit to these data appears wholly reasonable. The simple model fits provide support for the hypothesis that *I. hoferi*-induced mortality has been a significant factor in the dynamics of the stock.

Simulations using the disease dynamics model

Estimates of stock size and of fishing mortality using a conventional model to assess the simulated data are given in Fig. 8, together with the true values used to build the simulated data. This comparison affords some indication of how misleading such a misspecified model may be, and of what diagnostic residual patterns one might expect to observe. Figures 8*a*, 8*c*, and 8*e* suggest that one might expect (*i*) a decrease in the estimated stock size, (*ii*) an increase in the estimated fishing mortality, and (*iii*) a dome-shaped survey residual pattern. The estimated stock size is not very different from the simulated one, suggesting that little error is introduced in this respect, but the disease-induced mortality has been interpreted as an increase in fishing mortality. Although it is of course impossible

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Table 4. Parameter correlation matrices for the parasite components of two model parameterizations assuming either that infection rate depends on year (R(91) to R(94)) and age (R(91) to R(94)), or that infection rate depends on year-class (RC(83) to RC(92)) and on age RA(2) to RA(7).

Age and year-class model																
	RC (83)	RC (84)	RC (85)	RC (86)	RC (87)	RC (88)	RC (89)	RC (90)	RC (91)	RC (92)	RA (3)	RA (4)	RA (5)	RA (6)	RA (7)	MI
Year-class effects																
RC (83)	1.000															
RC (84)	0.976	1.000														
RC (85)	0.951	0.962	1.000													
RC (86)	–0.091	–0.087	–0.070	1.000												
RC (87)	–0.060	–0.060	–0.019	–0.202	1.000											
RC (88)	–0.002	–0.002	–0.002	–0.000	–0.001	1.000										
RC (89)	–0.114	–0.115	–0.113	0.131	–0.403	0.001	1.000									
RC (90)	–0.178	–0.182	–0.175	–0.524	–0.269	0.000	0.146	1.000								
RC (91)	–0.962	–0.973	–0.992	0.072	0.047	0.001	0.117	0.192	1.000							
RC (92)	–0.976	–0.995	–0.974	0.095	0.064	0.001	0.117	0.182	0.987	1.000						
Age effects																
RA (3)	–0.982	–0.996	–0.965	0.097	0.067	0.001	0.117	0.181	0.980	0.997	1.000					
RA (4)	–0.987	–0.991	–0.956	0.096	0.068	0.002	0.116	0.180	0.971	0.990	0.993	1.000				
RA (5)	–0.981	–0.973	–0.941	0.094	0.066	0.002	0.114	0.177	0.955	0.973	0.978	0.979	1.000			
RA (6)	–0.694	–0.678	–0.658	0.067	0.046	0.001	0.080	0.123	0.668	0.677	0.683	0.686	0.682	1.000		
RA (7)	–0.045	–0.046	–0.039	0.013	0.013	0.000	0.007	0.008	0.044	0.047	0.047	0.047	0.046	0.032	1.000	
Disease-induced mortality																
MI	–0.713	–0.716	–0.613	0.209	0.204	0.001	0.106	0.133	0.697	0.740	0.744	0.741	0.727	0.508	0.064	1.000

Age and year model											
	RY (91)	RY (92)	RY (93)	RY (94)	RA (2)	RA (3)	RA (4)	RA (5)	RA (6)	RA (7)	MI
Year effects											
RY (91)	1.000										
RY (92)	0.714	1.000									
RY (93)	0.881	0.736	1.000								
RY (94)	0.292	0.377	0.259	1.000							
Age effects											
RA (2)	–0.229	–0.278	–0.246	–0.109	1.000						
RA (3)	–0.715	–0.690	–0.749	–0.278	–0.004	1.000					
RA (4)	–0.844	–0.808	–0.836	–0.269	0.289	0.695	1.000				
RA (5)	–0.852	–0.541	–0.772	–0.140	0.196	0.704	0.820	1.000			
RA (6)	–0.424	–0.297	–0.367	–0.064	0.104	0.356	0.448	0.442	1.000		
RA (7)	–0.654	–0.524	–0.657	–0.176	0.188	0.606	0.734	0.767	0.229	1.000	
Disease-induced mortality											
MI	0.004	0.243	0.016	0.250	–0.021	0.115	0.185	0.476	0.264	0.401	1.000

here to distinguish trends in F or in stock abundance owing to model misspecification from real ones, Figs. 8*b*, 8*d*, and 8*f* from the base-line assessment show that the features observed in the misspecified fit are also present in the base-line assessment: increase in F, decrease in stock size, and dome-shaped residual pattern, the latter being particularly striking. Residuals at the other ages show similar patterns. This comparison also lends support to the hypothesis that the outbreak of *I. hoferi* has had a significant effect on the dynamics of the North Sea herring stock.

Parameterization of infection rate

Two model fits were calculated with infection rate R parameterized as an age and year effect (eq. 18) or as an age and year-class effect (eq. 19). The former parameterization is a representation of the hypothesis that the rate of infection by the disease is due to an endogenous factor dependent on the age of the fish, certain ages being more vulnerable by virtue of developmental stage or of migrations. The rate of infection is also hypothesized to be dependent on the year, there being a greater abundance of the infective agent in some years than in others. The second parameterization is a representation of the hypothesis that certain year-classes of fish became infected with the disease at a very early developmental stage, and that the symptoms of infection develop slowly until at a certain age in later life the symptoms become visible and significant mortality is incurred. The second hypothesis was formulated by Skagen (quoted in Anonymous 1995).

The error variances about the two models were similar and not distinguishable ($P(F) = 0.38$). A choice between the models

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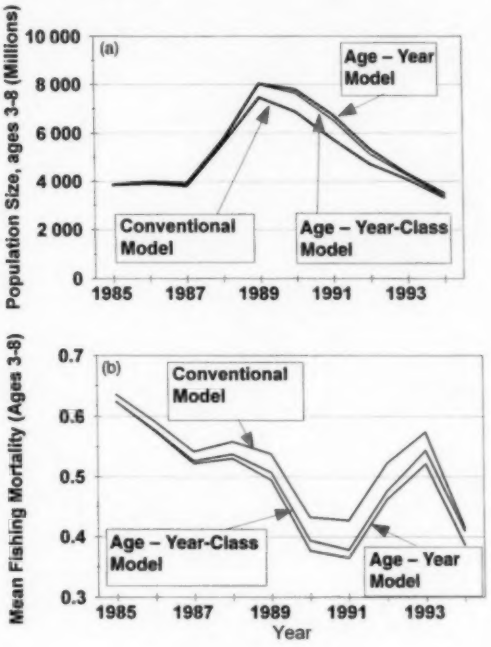
Fig. 9. Estimates of (*a*) population abundance and (*b*) fishing mortality obtained by using either a conventional stock assessment model (the base-line assessment) or the extended disease dynamics model (eqs. 15–19). Infection rate R is either assumed to be separable into a year component and an age component (age–year model) or into a year-class component and an age component (age – year-class model).



could not be made on this basis. However, the year-class and age model has much stronger correlations among the fitted parameters than the age and year model (Table 4), suggesting that the latter model should be more stable. The year and age formulation was therefore preferred on the grounds of numerical stability, simplicity, parameter parsimony, and slightly lower error variance. As a rather complex model is being fitted to few and noisy observations such considerations are important, but it is not possible to exclude the possibility that the year-class and age model may provide a better representation of the underlying process. However, it is not feasible to estimate the disease dynamics parameters of the year-class and age model with the observations available. Although the estimates of disease dynamics parameters for the two parameterizations were widely different, the estimates of population size and of fishing mortality were nevertheless similar. These estimates are plotted in Fig. 9, for comparison with the trends estimated using the conventional model in the base-line assessment. Choice of parameterization is not therefore critical to an assessment of the impact of the outbreak.

Observed and expected prevalences from the age and year parameterization are compared in Fig. 10. These coincide very well with the international acoustic survey but rather less well with the Norwegian autumn survey observations. As sample sizes in the international acoustic survey were substantially higher it is perhaps reasonable to expect a better fit to these observations. Despite the year and age parameterization of infection rate, predicted prevalences still apparently tend to follow certain cohorts: it is not necessary to use the year-class and age parameterization to account for this tendency.

Estimates of fitted parameters

Estimates of infection rates, fishing mortality, and the sizes of the infected and uninfected populations are given in Tables 5–7. These show that the infection rate was at a high level in 1991 and 1992, then declined to a lower rate in 1993. The infection rate estimated in 1994 was very low. Infection rates were low at younger ages and increased at ages 4 and 5. Estimates of the uncertainty in the parameters estimated directly in the nonlinear search (rather than implicitly in the structural model) are given in Table 8. As the confidence intervals are asymmetric this uncertainty is expressed as the standard deviation of the log-transformed parameter estimate, which approximates the coefficient of variation.

The MI parameter, representing the additional mortality rate of infected fish, was estimated to be 1.48 (coefficient of variation 0.21; 95% confidence limits 0.98–2.23). For comparison, Sindermann and Chenoweth (1993) reported on an experiment in which infected herring were held in aquaria. Severely infected individuals died within 1 month, and "virtually all" of the infected fish had died within 6 months. If half the fish had died within 3 months this would correspond to an MI of 2.77. It seems reasonable to expect that fish held in unavoidably stressful tank experiments should suffer a higher mortality rate than fish in the wild, hence the parameter estimate calculated here appears to be consistent with Sindermann and Chenoweth's experiments.

Density dependence of infection rate

Infection rate has been treated as an exogenously determined variable, although some previous authors have included an a priori assumption that infection rate is dependent on the numbers of infected (or infective) individuals in the population. However, other populations of fish in the North Sea also carry an *I. hoferi* burden (e.g., plaice (*Pleuronectes platessa*) and haddock (*Melanogrammus aeglefinus*); McVicar 1982, 1984), which probably release infective spores into the sea. Changes in climatic conditions may, under certain circumstances, facilitate the infection of herring by fish from another stock, such as perhaps the large blue whiting (*Micromesistius poutassou*) populations in the northeast Atlantic. As these populations are not included in the model and their relationships to the infection of North Sea herring are not understood, interannual changes in infection rate have been assumed to be determined by factors external to the dynamics of the disease in the herring stock. It may, in the future and with more data, prove possible to explore the dependence of infection rate on other variables such as the parasite burdens in the North Sea herring and in other fish populations. For these reasons it has been decided here to make no constraining assumption about the dependence of infection rates on other variables.

Implications for stock dynamics

Estimating parameters of disease dynamics allows an assessment of the importance of an outbreak for stock management. A simple approach to doing this is to simulate the development of the stock with estimated populations at the start of 1991 and

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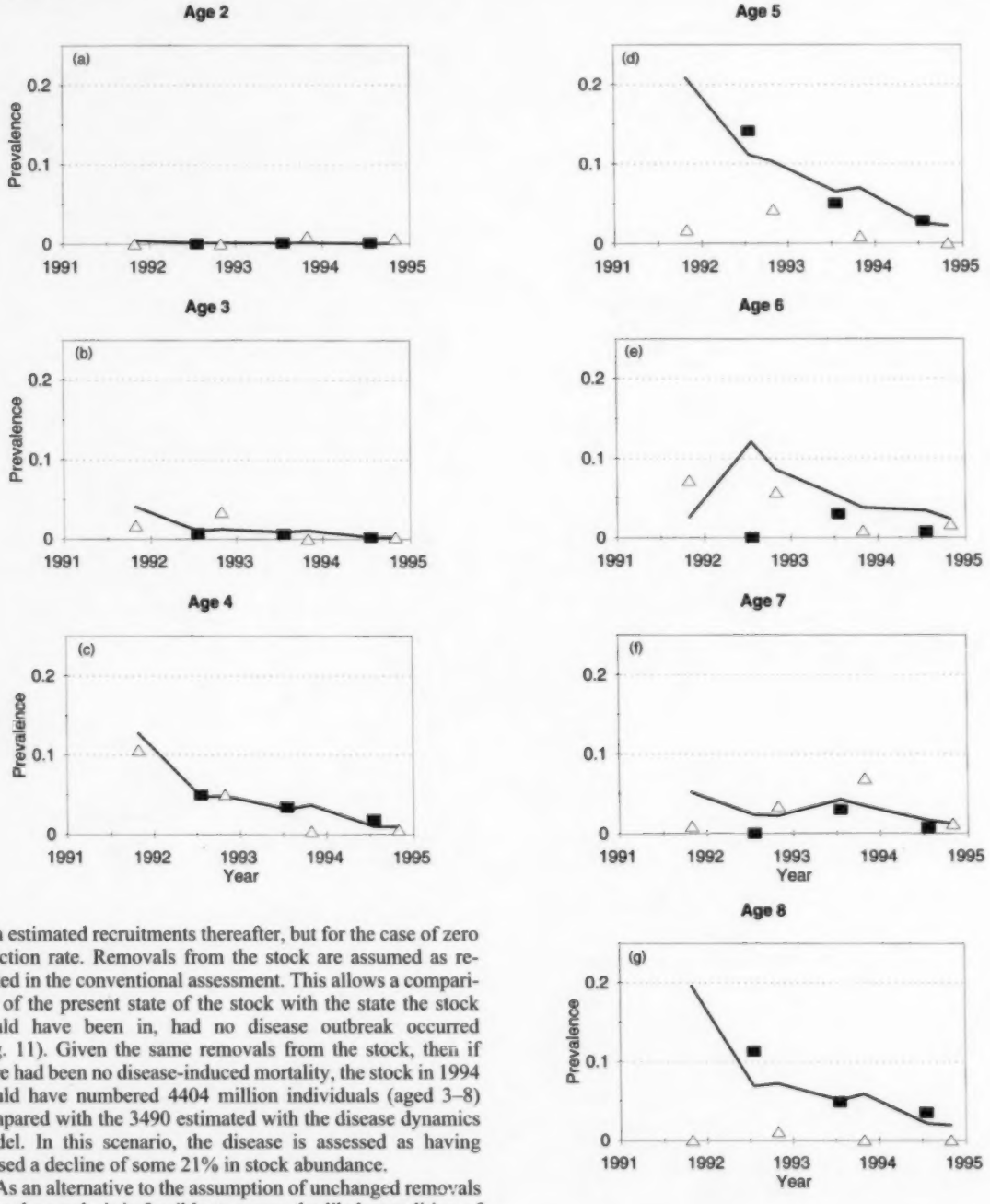
Fig. 10. Prevalence of parasite infection (proportion of fish with detectable disease) by age (in winter rings), as observed in the international acoustic surveys (solid squares), by the Norwegian bottom-trawl surveys (open triangles), and as predicted by the disease dynamics model (solid line).



with estimated recruitments thereafter, but for the case of zero infection rate. Removals from the stock are assumed as reported in the conventional assessment. This allows a comparison of the present state of the stock with the state the stock would have been in, had no disease outbreak occurred (Fig. 11). Given the same removals from the stock, then if there had been no disease-induced mortality, the stock in 1994 would have numbered 4404 million individuals (aged 3–8) compared with the 3490 estimated with the disease dynamics model. In this scenario, the disease is assessed as having caused a decline of some 21% in stock abundance.

As an alternative to the assumption of unchanged removals from the stock, it is feasible to assess the likely condition of the stock had there been no disease-induced mortality, but had fishing mortality remained unchanged (Fig. 12). Under these conditions, it is estimated that the total catches from 1991 to 1994 would have been 2 715 000 t compared with the 2 476 000 t reported taken, a difference of 9.6%. Population abundance would have been 3887 million compared with 3490 million estimated with the disease dynamics model, a difference of 11%. The impact of the *I. hoferi* outbreak has therefore been of the order of 10–20% on catches or on population size,

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Table 5. Fishing mortality (F, year^{-1}) estimated by fitting the extended year–age disease dynamics model.

	Year																			
Age	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
0	0.1470	0.1413	0.0955	0.0445	0.0835	0.1255	0.4830	0.3359	0.4004	0.2166	0.0844	0.0571	0.1486	0.1163	0.1250	0.0541	0.2116	0.2681	0.3025	0.2241
1	0.6833	0.2297	0.2837	0.1952	0.1626	0.1129	0.2848	0.2257	0.2532	0.1957	0.3776	0.3089	0.3350	0.5245	0.3891	0.4212	0.2100	0.2661	0.3002	0.2224
2	1.2773	1.3136	0.2031	0.0229	0.0920	0.3522	0.3231	0.2598	0.3033	0.3007	0.3971	0.4450	0.3901	0.3097	0.3345	0.3183	0.3859	0.4891	0.5519	0.4087
3	1.4931	1.3014	1.3135	0.0378	0.0628	0.4041	0.2635	0.5059	0.3233	0.4085	0.6578	0.5022	0.4759	0.3822	0.3325	0.2835	0.3534	0.4479	0.5054	0.3743
4	1.3286	1.6747	0.3449	0.0906	0.0828	0.2776	0.2881	0.2335	0.4331	0.5032	0.7123	0.5541	0.5449	0.5327	0.5079	0.3368	0.3767	0.4774	0.5387	0.3989
5	1.7599	1.3979	1.0452	0.0128	0.0450	0.2290	0.3750	0.1447	0.2561	0.5820	0.6229	0.5133	0.5595	0.5850	0.5524	0.4231	0.3375	0.4277	0.4826	0.3574
6	1.2307	0.8465	0.5158	0.0605	0.0095	0.0573	0.3527	0.1281	0.3175	0.3090	0.6678	0.6389	0.5501	0.5733	0.5453	0.3631	0.3946	0.5001	0.5643	0.4180
7	1.9136	1.3152	0.4510	0.0372	0.3216	0.0767	0.7493	0.1753	0.3337	0.5720	0.4621	0.6729	0.4713	0.5396	0.5157	0.4270	0.3471	0.4398	0.4963	0.3676
8	1.5155	1.2217	0.6572	0.0880	0.1342	0.2355	0.4234	0.2722	0.3542	0.4498	0.6161	0.5702	0.5279	0.5670	0.5119	0.4268	0.3767	0.4774	0.5387	0.3989
9	1.5155	1.2217	0.6572	0.0880	0.1342	0.2355	0.4234	0.2722	0.3542	0.4498	0.6161	0.5702	0.5279	0.5670	0.5119	0.4268	0.3767	0.4774	0.5387	0.3989

Table 6. Numbers of infected and uninfected fish estimated by fitting the extended year–age disease dynamics model (population sizes are estimated on 1 January).

Numbers of uninfected fish, N (millions)

	Year																			
Age	1975	1976	1977	1978	1979	1890	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1944	1995
0	3016	2828	4426	4708	10 633	16 761	37 812	64 519	61 726	53 164	81 102	103 900	90 962	44 581	39 756	37 433	40 703	83 176	69 521	42 006	55 135
1	7444	958	903	1480	1 657	3 598	5 439	8 582	16 964	15 216	15 749	27 420	36 100	28 843	14 600	12 609	13 046	12 119	23 403	18 899	12 351
2	843	1383	280	250	448	518	1 182	1 505	2 519	4 845	4 603	3 972	7 407	9 500	6 280	3 640	3 116	3 890	3 417	6 376	5 566
3	361	174	275	169	181	303	270	634	860	1 378	2 657	2 292	1 885	3 715	5 163	3 330	1 961	1 556	1 763	1 454	3 139
4	199	66	39	61	134	139	165	170	313	509	750	1 127	1 136	959	2 075	3 032	2 053	1 035	795	852	819
5	72	48	11	25	50	111	95	112	122	184	279	333	586	596	509	1 130	1 959	966	539	392	517
6	24	11	11	4	22	43	80	59	88	85	93	135	180	303	300	265	670	792	502	268	248
7	11	6	4	6	3	20	37	51	47	58	57	43	65	94	188	158	167	387	428	255	160
8	5	1	2	2	5	2	17	16	39	31	30	32	20	36	50	83	93	96	219	229	160
9	2	1	1	1	3	6	6	13	20	37	39	34	34	29	33	45	76	105	113	175	246

Numbers of infected fish, NI (thousands)

	Year			
Age	1991	1992	1993	1994
2	0	0	0	0
3	0	6 977	2 115	1 633
4	0	47 902	10 464	9 901
5	0	155 918	27 661	15 888
6	0	232 959	55 409	20 682
7	0	11 122	32 293	8 291
8	0	57 380	4 888	7 391

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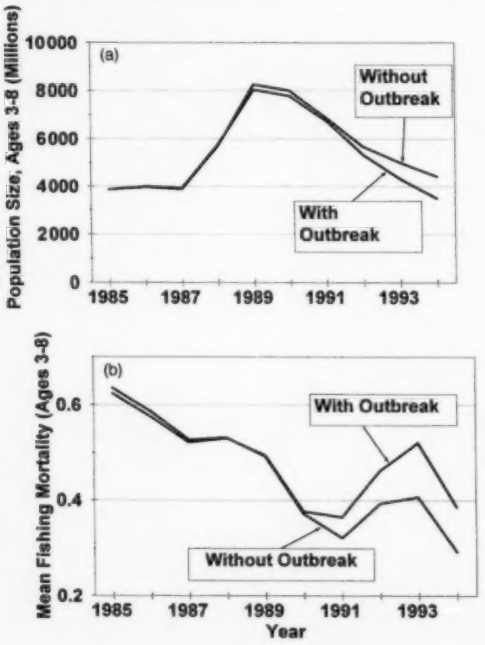
Table 7. Predicted prevalence at the time of the international acoustic and Norwegian autumn trawl surveys.

Age	Year			
	1991	1992	1993	1994
International acoustic survey				
2		0.09	0.08	0.02
3		1.06	0.86	0.20
4		4.75	3.17	0.99
5		11.13	6.51	2.57
6		12.03	5.14	3.40
7		3.36	4.25	1.61
8		6.87	5.04	2.16
Norwegian autumn trawl survey				
2	0.41	0.11	0.10	0.02
3	4.05	1.23	1.06	0.22
4	12.72	4.84	3.67	0.95
5	20.80	10.31	6.96	2.20
6	2.56	8.59	3.77	2.33
7	5.18	2.24	3.46	1.18
8	19.58	7.21	5.81	1.90

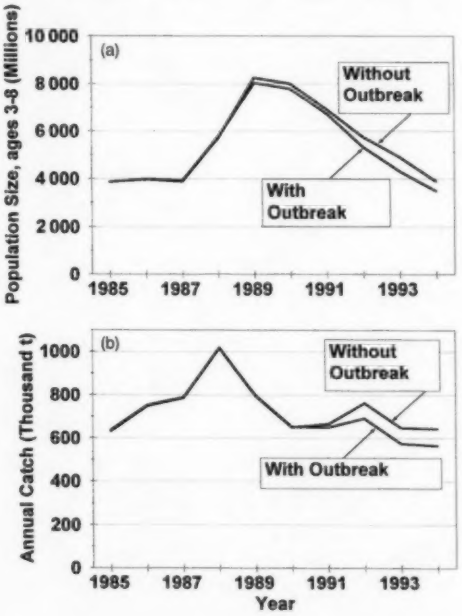
Fig. 11. Impact of the *I. hoferi* outbreak. (*a*) Population abundance and (*b*) fishing mortality estimated using the extended model, compared with a simulation in which infection rates are assumed to be zero but the same yield was harvested.



depending on assumptions about exploitation in the event that no disease had occurred. Although significant and measurable, such losses from the stock are less severe than might have been expected from the Gulf of St. Lawrence example, and are substantially less than catastrophic.

The model in its present form can be used to assess the significance of the impact of a historical outbreak of a disease

Fig. 12. Impact of the *I. hoferi* outbreak. Population abundance and fishing mortality estimated using the extended model, compared with a simulation in which infection rates are assumed zero but fishing mortalities are assumed to be the same as those estimated in the model fit. (*a*) Population abundance. (*b*) Annual catches.



on the size and potential yield of a stock. However, the model does not yet extend to a consideration of the origins of an outbreak, nor can it be used to propose management strategies to improve yield despite the vulnerability of an exploited stock to a lethal disease. This is because the infection rate R is treated as exogeneously determined; the causes of the outbreak remain unknown. In the case of North Sea herring, it seems unlikely that it will be possible to assess the factors determining the infection rate, whether they be exogenous or endogenous, until it has been possible to monitor several further disease outbreaks in some detail. Formulation of a model such as that presented here does, however, allow estimation of the infection rate, which must be a first step towards eventually determining the causative factors of the disease outbreak.

Conclusions

The simple model used here showed that the fit of the catch and survey observations to the conventional assessment model could be improved significantly by assuming that the stock suffered an additional mortality proportional to the observed prevalence of diseased individuals in the population. Furthermore, the simulation experiment indicated that if a conventional model were used to estimate population size and fishing mortality in a stock that has undergone an outbreak of a lethal disease, one should expect to find a dome-shaped survey residual pattern with time, an increase in fishing mortality, and a decline in stock abundance. All three features are found in the most recent conventional assessments of the North Sea herring stock. These findings indicate the need for a more formal

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Table 8. Estimates of parameters found in the nonlinear search.

		Estimate	Coefficient of variation
Reference fishing mortality by year	F (1991)	0.376 7	0.159
	F (1992)	0.477 4	0.157
	F (1993)	0.538 7	0.168
	F (1994)	0.398 9	0.197
Selection by age	S (0)	0.561 6	0.785
	S (1)	0.557 4	0.231
	S (2)	1.024 5	0.185
	S (3)	0.938 2	0.185
	S (4)	1.000 0	Constrained
	S (5)	0.895 9	0.176
	S (6)	1.047 7	0.170
	S (7)	0.921 4	0.172
	S (8)	1.000 0	Constrained
Uninfected populations in 1994 by age	N (0,1994)	42 005 870	0.229
	N (1,1994)	18 898 563	0.186
	N (2,1994)	6 376 442	0.165
	N (3,1994)	14 54 446	0.167
	N (4,1994)	852 278	0.171
	N (5,1994)	391 671	0.181
	N (6,1994)	267 762	0.191
	N (7,1994)	255 077	0.210
	N (8,1994)	229 496	0.218
Uninfected populations at age 8 by year	N (8,1991)	93 019	0.278
	N (8,1992)	95 624	0.226
	N (8,1993)	218 966	0.206
Recruitment in 1995	N (0,1995)	55 134 644	0.290
Survey index catchabilities			
Larval index of spawning stock	QL	0.001 050 1	0.087
International acoustic survey, by age	QA (2)	1.049 4	0.277
	QA (3)	1.037 6	0.278
	QA (4)	1.103 9	0.278
	QA (5)	1.293 9	0.280
	QA (6)	1.373 4	0.280
	QA (7)	1.314 4	0.283
	QA (8)	1.285 1	0.284
	QA (9)	1.100 8	0.285
International bottom trawl survey, by age	QB (1)	0.000 118 22	0.071
	QB(2)	0.000 152 85	0.155
	QB(3)	0.000 100 73	0.156
	QB(4)	0.000 062 457	0.157
	QB(5)	0.000 033 947	0.158
International Methot–Isaacs–Kidd survey	QM	0.000 003 1281	0.073
Disease-related parameters			
Infection rate by year	RY (1991)	0.438 5	0.459
	RY (1992)	0.117 6	0.483
	RY (1993)	0.110 1	0.426
	RY (1994)	0.020 3	1.250
Infection rate by age	RA (2)	0.019 5	1.533
	RA (3)	0.195 8	0.519
	RA (4)	0.633 4	0.460
	RA (5)	1.067 3	0.508
	RA (6)	0.123 1	0.877
	RA (7)	0.251 5	1.040
Disease-induced mortality of infected fish	MI	1.482 0	0.208

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description of the disease dynamics, and such a model has been developed and fitted. This new extended disease dynamics model has explained satisfactorily the observations of stock abundance, prevalence of the disease, and catches from the stock. The pattern of prevalence could be explained well on the basis of a year and age effect; it was not necessary to invoke a year-class effect. The model allowed an assessment of the impact of the disease on the stock and was sufficiently flexible to allow calculation of the consequences of alternative assumptions about the dynamics of the *I. hoferi* outbreak.

The fitted models indicated that the North Sea herring stock suffered an outbreak of the *I. hoferi* disease that caused statistically significant additional mortality to the stock. This outbreak was first detected in 1991, declined to a quarter of the 1991 level in 1992 and 1993, and had almost disappeared by 1994. Infection rates were highest at ages 3–5. Infected fish suffered an additional mortality rate more than 10 times higher than that of uninfected fish. Indications for management are that the conventional assessment calculation returns estimates of current-year stock size that are approximately correct, but fishing mortality is overestimated and historical stock sizes are underestimated.

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Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia

S.C. Johnson, R.B. Blaylock, J. Elphick, and K.D. Hyatt

Abstract: The occurrence of the marine ectoparasitic copepod *Lepeophtheirus salmonis* and the prevalence of lesions caused by its feeding activities were monitored on sockeye salmon (*Oncorhynchus nerka*) adults returning to the Sproat and Stamp rivers through Alberni Inlet, British Columbia, in 1990 and 1992–1993. All sockeye examined were infected with *L. salmonis* and had higher intensities of infection than previously reported. The presence of high numbers of early developmental stages of *L. salmonis* suggests a high rate of infection for sockeye in coastal waters. Lesions attributable to *L. salmonis* ranged from minor skin discoloration to large open lesions that exposed the musculature. In 1990, when escapement into these river systems was delayed, sockeye holding in the inlet developed severe lesions and suffered high mortalities. High percentages of fish with open lesions entered both river systems in 1990, but few fish with open lesions were observed on the spawning grounds, suggesting additional prespawning mortality. In 1992 and 1993, when escapement patterns were more normal than in 1990, the severity of lesions owing to *L. salmonis* was reduced and no mortalities were observed. Throughout the study, fish in the Sproat River escapement had more severe lesions than those in the Stamp River escapement.

Résumé : Nous avons surveillé l'occurrence du copépode marin ectoparasite *Lepeophtheirus salmonis* et la prévalence des lésions causées par son activité alimentaire chez des adultes de saumon rouge (*Oncorhynchus nerka*) retournant aux rivières Sproat et Stamp en passant par l'inlet Alberni (Colombie-Britannique) en 1990 et en 1992–1993. Tous les saumons rouges examinés étaient infestés par *L. salmonis*, et présentaient des infestations plus intenses que ce qui avait été observé jusque-là. La présence d'un nombre important de parasites aux premiers stades du développement indique probablement un fort taux d'infestation des saumons dans les eaux côtières. Les lésions attribuables à *L. salmonis* allaient de simples altérations de la couleur de la peau à de grandes lésions ouvertes qui exposaient la musculature. En 1990, alors que la remonte vers ces cours d'eau a été retardée, les saumons rouges qui demeuraient dans l'inlet ont subi de graves lésions et une forte mortalité. Des pourcentages importants de poissons portant des lésions ouvertes ont pénétré dans les deux réseaux en 1990, mais rares étaient les individus atteints de lésions observés sur les frayères, ce qui permet de penser qu'il se produit une mortalité additionnelle avant la fraye. En 1992 et 1993, alors que les patrons des échappées étaient plus normaux qu'en 1990, la gravité des lésions dues à *L. salmonis* a baissé, et on n'a observé aucune mortalité. Tout au long de l'étude, les poissons de l'échappée de la rivière Sproat présentaient des lésions plus graves que ceux de l'échappée de la rivière Stamp.
[Traduit par la Rédaction]

Introduction

The marine ectoparasitic copepod *Lepeophtheirus salmonis* (Krøyer), often referred to as the salmon or sea louse, is commonly found on most species of wild and sea-farmed salmonids of the genera *Oncorhynchus*, *Salmo*, and *Salvelinus* in the northern hemisphere (Kabata 1979, 1988; Wootten et al. 1982; Pike 1989). The life cycle is direct, consisting of five phases and 10 stages. These include two free-swimming naupliar stages, one free-swimming infectious copepodid stage, four attached chalimus stages, two preadult stages, and an adult stage (Johnson and Albright 1991*a*). Attached copepodids,

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chalimus larvae, preadults, and adults feed on host mucus, skin, and blood (Kabata 1974; Brandal et al. 1976).

Epizootics of *L. salmonis* commonly occur in sea-farmed salmonids, causing serious disease and high mortality rates if untreated (Brandal and Egidius 1979; Wootten et al. 1982; Pike 1989). This disease is characterized by extensive areas of skin erosion and hemorrhaging on the head and back and a distinct area of erosion and subepidermal hemorrhage in the perianal region (Brandal and Egidius 1979; Wootten et al. 1982). Death may be caused by secondary bacterial infections (e.g., vibriosis), fungal infections acquired when fish return to fresh water, or, in severe cases, osmotic stress (Wootten et al. 1982).

Lepeophtheirus salmonis is common on wild salmonids but generally present only in low abundance and responsible for only minor host tissue damage (abrasion, dark coloration, and hemorrhages of the perianal region) (Wootten et al. 1982; Nagasawa 1987; Nagasawa et al. 1993; Nagasawa and Takami 1993). However, in certain situations, *L. salmonis* may be associated with disease conditions. Lesions that range in severity from discoloration to complete removal of the skin from the heads and backs of wild Atlantic salmon (*Salmo salar*) have been reported (Calderwood 1906; Huntsman 1918; White 1940, 1942) and attributed to sea louse feeding (Huntsman 1918; White 1940, 1942). In addition to forming foci for secondary fungal infections (Huntsman 1918), the presence of severe sea louse lesions coupled with high water temperatures were reported to cause the death of Atlantic salmon in Moser River, Nova Scotia, over the period of 1939–1942 (White 1940, 1942). Recently, high levels of mortality in wild postsmolt sea trout (*Salmo trutta*) in waters off the west coast of Ireland have been attributed to sea lice (Tully et al. 1993*a*, 1993*b*). These infections resulted in extensive tissue damage to the fins and dorsal body surfaces. Gilhousen (1989) reported that sockeye salmon caught in the Fraser River, British Columbia, were only lightly infected with *L. salmonis* during July and August of 1971. However, during September and October of the same year, he reported extensive parasitism by sea lice that resulted in the development of large abraded areas and open wounds, especially behind the dorsal fin and in the perianal region.

This paper documents the dynamics and severity of the sea louse disease outbreak in sockeye salmon stocks of Alberni Inlet, British Columbia, that occurred in 1990 when migration into the Somass River was delayed, as well as the dynamics and severity of sea louse disease in these stocks in years with normal migration patterns (1992 and 1993). These data provide the baseline information necessary to assess the causes and the impact of future sea louse disease outbreaks in these and other wild stocks of salmonids.

Materials and methods

Study area, stocks, and sampling

Alberni Inlet is a typical fjord situated on the southwest coast of Vancouver Island opening into the Pacific Ocean through Barkley Sound. Oceanographic conditions of the inlet are given in Morris and Leaney (1980). Within the Alberni watershed, Sproat, Great Central, and Henderson lakes support sockeye stocks (Hyatt and Steer 1987). The Stamp and Sproat rivers drain Great Central and Sproat lakes, respectively. These rivers join to form the Somass River, which drains into the head of Alberni Inlet. Sampling was conducted at fishways located approximately 0.5 km below the outlet of Sproat Lake and at a dam that forms the outlet of Great Central Lake. Details concerning sockeye abundance, annual run timing, and the fisheries they support are found in Hyatt and Steer (1987).

In 1990, samples of sockeye from Alberni Inlet were caught using a commercial gill-net boat equipped with a 11-cm mesh gill net. In 1991–1993, samples were obtained from commercial seine and gill-net boats. In all years, samples from the fishways of the Sproat and Stamp rivers were caught in traps that form part of the fishways. Beach seining was used to collect samples from the spawning grounds within Sproat and Great Central lakes in 1990.

Data on the abundance of sea lice were obtained from samples of fish collected by gill net (1990 and 1991), longline, trawl, or seine (1992). Upon landing, fish were placed immediately in individual plastic bags and stored frozen prior to examination. Whole fish were examined under a dissecting scope. All sea lice were counted and the general developmental stage (copepodid, chalimus, preadult, or adult) was noted. Preadult and adult stages were identified to species.

The terms prevalence and abundance as used in this paper are defined by Margolis et al. (1982). The term escapement refers to those fish that escape capture and enter the the lake systems.

Lesion classification

Data on the prevalence and severity of sea louse lesions and other wounds and scars were collected in the fall and winter of 1990. Samples obtained from the fishways of the Sproat and Stamp rivers in September were classified as (*i*) unmarked, (*ii*) open lesions (dorsal lesions extending through to the dermis, exposing underlying musculature), and (*iii*) other marks (this category includes lesions in which the dermis was not breached, as well as fungal disease, predator wounds, and marks attributed to commercial fishing activities). Lesions on samples collected from the spawning grounds in the fall and winter were classified as having mild (one open lesion), moderate (two or three open lesions), or severe (more than three open lesions) external damage.

In the summer and fall of 1992 and 1993, data on the prevalence and severity of sea louse lesions were collected from test fishery samples obtained from Alberni Inlet by gill net or seine and from escapement samples from the fishways of the Sproat and Stamp rivers. Lesions attributable to sea lice occurred on the head, at the base of and posterior to the dorsal fin, and in the perianal region, and were ranked as follows. (A) Posterior to dorsal fin, base of dorsal fin, and head: rank 0, unmarked; rank 1, grazing evident, with discoloration of skin surface, and in some cases mild descaling and hemorrhaging; rank 2, early white lesion, with partial removal of epidermis and scales resulting in patches of grayish to whitish necrotic tissue; rank 3, white lesion, with epidermis and scales removed, lesions covered with continuous whitish necrotic tissue, and no breaks in skin exposing musculature (Fig. 1A); rank 4, early open lesion, white lesion with small (1–2 mm) point lesions open to musculature; and rank 5, open lesion, with epidermis and dermis removed exposing musculature (Fig. 1B). (B) Perianal region: rank 0, unmarked; rank 1, grazing evident, characterized by discoloration of skin surface and in some cases blood seepage from the scale pockets; rank 2, mild descaling and hemorrhaging; rank 3, extensive discoloration, descaling, and hemorrhaging (Fig. 1C); rank 4, early open lesion, with extensive descaling and hemorrhaging with small point lesions (1–2 mm) open to the musculature; and rank 5, open lesion, with epidermis and dermis removed, exposing musculature (Fig. 1D).

An overall lesion score (LS) of sea louse wounds was calculated for each fish by summing the rank for each of the four regions above. The LS could have a value of 0–20, with 20 representing the most severe damage.

Microbiology and histology

Because of high levels of mortality in 1990, samples of gill, kidney,

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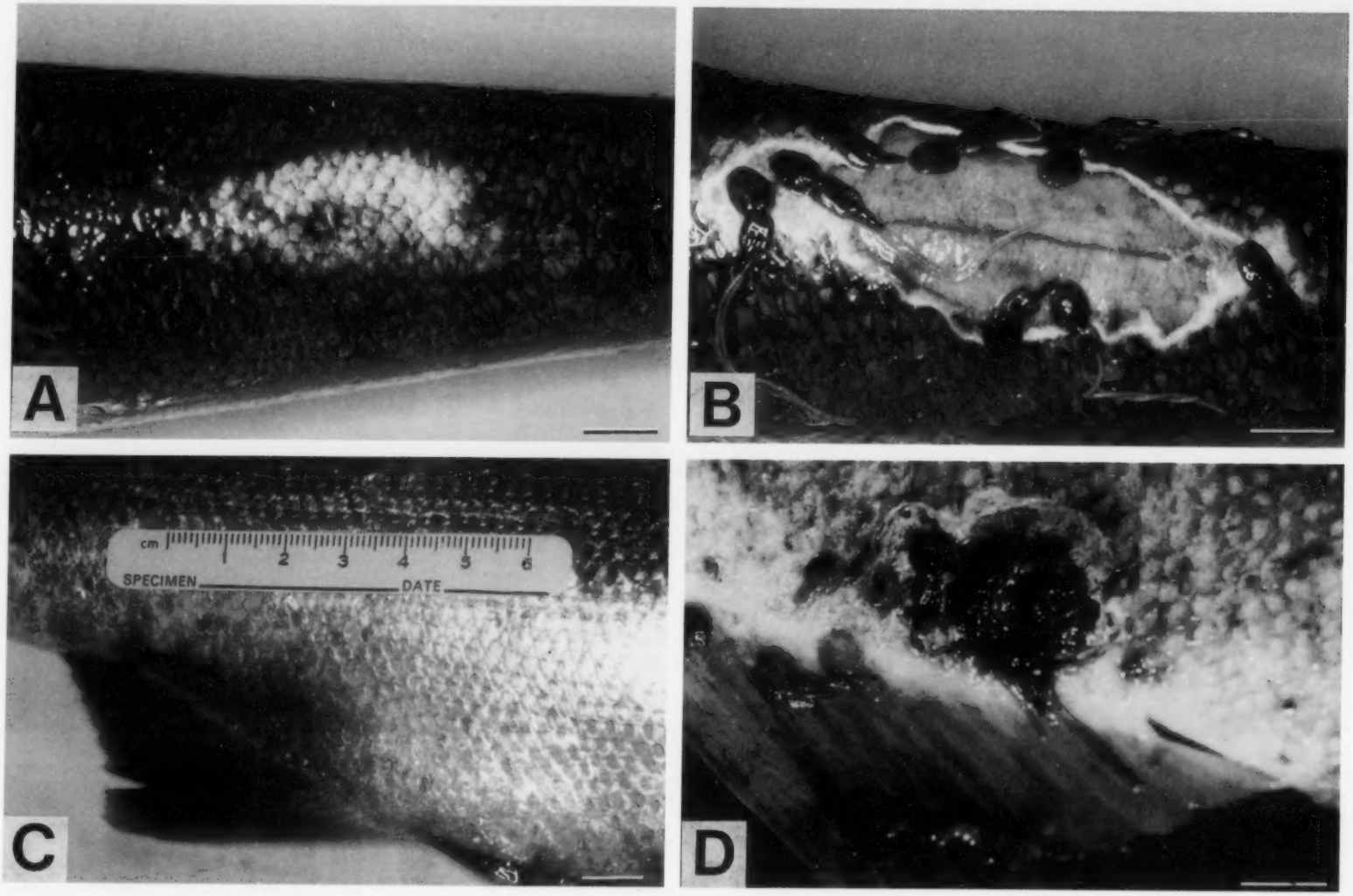
Fig. 1. Lesions caused by *Lepeophtheirus salmonis* on sockeye salmon (*Oncorhynchus nerka*). (A) Rank 3, white lesion posterior to the base of the dorsal fin. (B) Rank 5, open lesion posterior to the base of the dorsal fin. Note the exposed musculature and several adult female *L. salmonis* feeding within the lesion. (C) Rank 3, perianal region with extensive discoloration, descaling, and hemorrhaging. (D) Rank 5, perianal region with open lesion. Note the exposed musculature. Scale bars = 1.0 cm.



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spleen, and pyloric ceca were collected from freshly killed sockeye adults caught in the inlet and rivers and assayed for the presence of bacteria and viruses according to standard techniques (Anonymous 1984). Bacteriological samples were streaked on plates of TSA and TSA plus salt, incubated in air at 20°C, and checked for growth after 2 and 8 days. Bacterial isolates as well as smears of kidney and gut were examined by employing standard biochemical techniques (Anonymous 1984). Virological samples were homogenized in Earle's balanced salt solution and centrifuged. Supernatants were collected, serially diluted, inoculated onto an epithelial papilloma cyprinid cell line, and monitored for 2–3 weeks for the formation of plaques. Samples of external lesions, gill, kidney, pyloric ceca, lower intestine, liver, and spleen were also collected from freshly killed individuals, fixed in Davidson's solution, and dehydrated through to 100% alcohol. Tissues were wax embedded, cut to a thickness of 5 μm, and stained with hematoxylin and eosin. Sections of lesions from fish collected in fresh water were also stained with periodic acid Schiff's and Gram's stains.

Statistical analysis

The distribution of LS on fish collected from Alberni Inlet between years (1992 and 1993) was compared using Pearson's χ^2 statistic. Lesion scores on the escapement samples from Sproat and Stamp rivers were compared both between years and between systems using Pearson's χ^2 statistic. The effects of host sex, hypural length, and sea age on lesion sums were investigated using Spearman's rank correlation.

Results

Lepeophtheirus salmonis was the dominant parasitic copepod collected during this study, although a few *Caligus clemensi* chalimus larvae and adults were found. In all samples, the prevalence of *L. salmonis* was 100% and all developmental stages, except for the free-swimming naupliar stages, were found (Table 1). Sockeye collected in September 1990 were the most heavily infected, with a mean of 300 *L. salmonis*/fish (range 49–1372). Observation of copepods made in the field on other dates in 1990 estimated up to approximately 300 preadults and adults present. The mean abundance of *L. salmonis* in 1991 and 1992 was considerably lower, ranging from 7.4 to 77.1 copepods/fish. Sockeye collected offshore, near the Queen Charlotte Islands, and from the surf zone at the mouth of Alberni Inlet in 1992 had mean abundances of 22.5 and 71.7 copepods/fish, respectively (Table 1). Copepodids and chalimus larvae were found most commonly on the body (68.4 and 52.6%, respectively), followed by the fins (29.0 and 43.3%, respectively) and then the gills (2.6 and 4.0%, respectively).

Alberni Inlet

In early to mid-September 1990, moribund sockeye with heavy infections of *L. salmonis* were commonly observed swimming at the surface of Alberni Inlet. Almost 100% of sockeye collected from Alberni Inlet during this time were heavily infected with *L. salmonis* and had lesions caused by sea louse feeding. In 87% of these fish the ulcers extended through the dermis, exposing the underlying musculature. These ulcers occurred most commonly on the dorsal and lateral surfaces of the head and the dorsal surface posterior to the dorsal fin. In addition, most fish had multiple shallow ulcers, epithelial abrasions, and scale loss that could not be attributed to netting.

Table 1. Abundance of *Lepeophtheirus salmonis* developmental stages on maturing sockeye salmon (*Oncorhynchus nerka*) in Alberni Inlet and offshore waters.

Date and locality	Sampling method	Sample size	Copepodids		Chalimus larvae		Preadults		Adults		Total copepods	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Sept. 17, 1990,[a] Alberni Inlet	Gill net	9	95.4±210.5	0–633	141.2±223.2	12–721	17.6±17.8	4–63	45.8±27.6	9–85	300.0±424.8	49–1372
July 23, 1991,[b] Alberni Inlet	Gill net	15	1.1±2.4	0–9	9.6±10.6	0–40	0.6±0.7	0–2	15.5±10.7	1–35	26.9±13.1	3–59
June 28, 1992, Alberni Inlet mouth	Trawl	3	11.3±11.0	5–24	15.3±0.6	15–16	12.7±5.5	7–18	32.3±11.0	25–45	71.7±14.6	55–82
July 1, 1992, Alberni Inlet	Seine	21	0.6±1.6	0–6	2.3±2.4	0–6	1.0±1.3	0–5	3.5±2.7	0–9	7.4±4.7	0–18
July 7, 1992, Alberni Inlet	Seine	22	28.5±41.5	0–180	28.6±30.4	4–106	5.3±8.2	0–39	14.2±11.7	0–41	77.1±61.1	15–220
July 19, 1992, offshore	Longline	2	3.5±5.0	0–7	1.0±1.4	0–2	6.0±2.8	4–8	12.0±2.8	10–14	22.5±3.5	20–25
July 20–21, 1992, Alberni Inlet	Seine	33	0.2±0.5	0–2	10.7±13.1	0–58	0.6±0.8	0–2	4.9±3.4	1–17	16.5±13.6	3–64

[a]Some sample bags were torn, which may have resulted in copepod loss.
[b]Body of fish was not examined for copepodids and chalimus larvae.

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Fig. 2. Distribution of lesion scores (see text) for Alberni Inlet sockeye (*Oncorhynchus nerka*) collected in July and August of 1992 and 1993. Examined fish were from test fishery samples collected in July and August of each year.

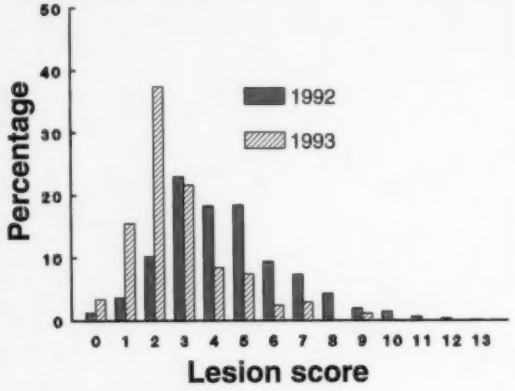
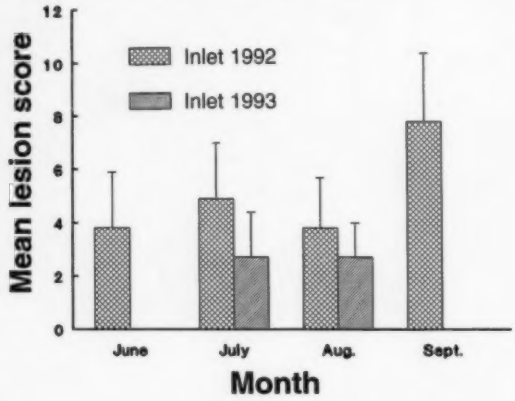


Fig. 3. Mean (+SD) monthly lesion scores for Alberni Inlet sockeye (*Oncorhynchus nerka*) collected in 1992 and 1993.



Scale removal, blood seepage, and tissue darkening occurred in the perianal region of most fish.

Examination of tissue samples collected for bacteriology and virology revealed no disease organisms that might account for the morbidity. Tissue sections of fins where chalimus larvae were feeding revealed mild epithelial hyperplasia associated with the frontal filaments, epidermal erosion at the feeding sites, and a small amount of inflammatory infiltrate. Tissue sections of the large open lesions revealed a progressive deepening of the lesions toward the center where both the epithelium and dermis were removed and the underlying muscle was exposed. Congestion occurred in the blood vessels of the exposed muscle, but little inflammation was observed. A small amount of filamentous bacteria was present on the surface of approximately 15% of the lesions and within the tissue (dermis or muscle) immediately beneath it.

Histological examination of the gills revealed no abnormalities. Examination of the internal organs revealed only a mild inflammatory response associated with a normal level of infection (5–20 worms) by the nematode *Philonema oncorhynchi*.

Table 2. Percentage of Alberni Inlet sockeye salmon (*Oncorhynchus nerka*) with lesions (see text) attributed to the feeding activities of *Lepeophtheirus salmonis* by body region and month for 1992 and 1993.

Lesion position and month	Lesion ranking						No. of fish examined
	0	1	2	3	4	5	
Posterior to dorsal fin							
June 1992	6.8	39.5	31.1	19.2	2.3	1.1	177
July 1992	0.5	15.1	58.4	17.9	1.8	6.3	397
August 1992	10.7	32.6	26.5	20.8	0.7	8.7	298
September 1992	0	11.2	14.4	54.4	4.8	15.2	125
July 1993	22.5	55.7	12.1	7.1	1.1	1.4	280
August 1993	7.0	70.0	8.0	10.0	1.0	4.0	100
Base of dorsal fin							
June 1992	93.2	2.3	2.3	1.1	0	1.1	177
July 1992	63.0	24.2	8.8	3.8	0	0.3	397
August 1992	48.0	46.0	3.4	2.0	0	0.7	298
September 1992	0.8	87.2	2.4	5.6	0.8	3.2	125
July 1993	72.9	25.7	1.1	0.4	0	0	280
August 1993	76.0	23.0	0	1.0	0	0	100
Head							
June 1992	97.7	0.6	1.7	0	0	0	177
July 1992	99.7	0	0	0	0	0.3	397
August 1992	94.3	0.3	1.7	2.3	0.3	1.0	298
September 1992	18.4	40.8	8.8	13.6	6.4	12.0	125
July 1993	99.3	0.4	0	0.4	0	0	280
August 1993	100.0	0	0	0	0	0	100
Perianal							
June 1992	9.6	44.6	0	44.6	0	1.1	177
July 1992	0.8	48.6	0	46.9	0.5	3.3	397
August 1992	2.3	86.9	9.1	1.3	0	0.3	298
September 1992	0.8	68.6	0.2	24.0	2.4	3.2	125
July 1993	9.6	67.1	12.9	8.9	0.4	1.1	280
August 1993	1.0	95.0	4.0	0	0	0	100

Lesions similar to those seen in 1990 occurred on fish examined in 1992 and 1993, although their severity was not as great, and moribund and dead fish were not observed. Fish collected in July and August of 1992 had higher LS than fish collected in July and August of 1993 (Pearson's χ^2, $P < 0.001$; Figs. 2 and 3), and in 1992 the LS increased over time from approximately 4 in June to approximately 8 in September (Fig. 3). In both 1992 and 1993 the severity of lesions at the base of the dorsal fin, on the head, and in the perianal region was low, with the majority of fish being either unmarked (rank 0) or only mildly damaged (rank 1) in these regions (Table 2). With respect to the region posterior to the dorsal fin, there were more severe lesions (rank 2 or higher) in 1992 than in 1993 (Table 2).

Escapement samples

In September 1990, there were higher percentages of fish with open or severe lesions in the Sproat River than in the Stamp River (Tables 3 and 4). From 47 to 100% of the Sproat fish had open lesions compared with 13 to 34% of the Stamp fish (Table 3). The severity of lesions on fish in the escapement samples collected from both rivers in 1992 and 1993 was less than in 1990, and the severity of lesions, as measured by the mean monthly LS, was greater in 1992 than in 1993 (Fig. 4).

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Table 3. Percentage of sockeye salmon (*Oncorhynchus nerka*) from the Sproat and Stamp rivers with dorsal lesions and (or) other marks in September 1990.

Date (in 1990)	Location	Sampling method	*n*	Unmarked	Open lesions	Other marks
Sept. 12	Sproat	Observer	ns		100	
Sept. 12	Stamp	Observer	143	79	13	8
Sept. 13	Sproat	Trap	50	2	78	20
Sept. 13	Stamp	Trap	50	10	34	56
Sept. 17	Sproat	Observer	51	30	47	23
Sept. 17	Stamp	Observer	195	65	24	11
Sept. 24	Stamp	Observer	86	53	19	28

Note: Lesions were ranked as follows: unmarked; open lesions, dorsal lesion extending through the dermis exposing underlying musculature; other marks, dorsal lesions in which the dermis was not breached, fungal lesions, predator wounds, and marks attributed to commercial fishing. ns, sample size not specified.

For both river systems, the percentage of open lesions (rank 5) also was higher in 1992 than in 1993 (Table 5). In both 1992 and 1993, fish from the Sproat River had higher percentages of the higher LS and higher mean monthly LS than fish from the Stamp River (Figs. 4 and 5). Open lesions occurred behind the dorsal fin in 21.8 and 19.2% of the fish examined from the Sproat River in 1992 and 1993, respectively. In the Stamp River, open lesions occurred behind the dorsal fin in 10.4 and 4.4% of the fish in 1992 and 1993, respectively. For both river systems and years, the severity of lesions at the base of the dorsal fin, on the head, and in the perianal region was generally low (rank 0 or 1) (Table 5).

The majority of fish collected on the spawning grounds in both Sproat and Great Central lakes in October and November of 1990 were unmarked (Table 4). For the Sproat Lake fish, between 0 and 14% had a single open dorsal lesion, and between 0 and 4% had two or more open dorsal lesions. For the Great Central Lake fish, between 8 and 15% had a single open dorsal lesion, and between 0 and 7% had two or more open dorsal lesions.

For both the Sproat and Stamp rivers in 1992, there were significant correlations between LS and hypural length (Spearman rank; $P < 0.01$, $n = 212$ for Sproat River; $P < 0.001$, $n = 273$ for Stamp River) and sea age (Spearman rank; $P < 0.001$, $n = 184$ for Sproat River; $P < 0.001$, $n = 53$ for Stamp River). In addition, there was a marginally significant correlation (Spearman rank; $P < 0.04$, $n = 273$) with host sex in the Stamp River, with males having a marginally higher LS than females. In 1993 there were no significant correlations between LS and host sex, hypural length, or sea age in either system.

Discussion

All sockeye examined for the presence of sea lice were infected with *L. salmonis* and had higher levels of infection than previously reported for sockeye. Prevalences between 0.6 and 12.5% and abundances between 0.02 and 0.32 copepods/fish have been reported previously for sockeye collected with longlines on the high seas of the North Pacific Ocean (Nagasawa 1987; Nagasawa et al. 1993; K. Nagasawa, National Research Institute of Far Seas Fisheries, Fisheries Agency of Japan, Shimizu, Japan, personal communication). However, Nagasawa's counts were based on either total adult or adult female *L. salmonis* only. The higher prevalences and intensities of infection reported here are due in part to our enumeration of the previously unreported earlier developmental stages and in part to the fact that our samples were taken in coastal waters. The presence of high numbers of early developmental stages (copepodids and chalimus larvae) suggests a high rate of infection by *L. salmonis* in coastal waters. However, our use of gill nets and seines to collect fish most likely resulted in an underestimation of the actual number of preadult and adult copepods. Nagasawa (1985) estimated that 77% more adult *L. salmonis* were lost because of net abrasion from gill-netted chum salmon (*Oncorhynchus keta*) than from those caught by longline.

The lesions seen in this study are the same as those reported from wild and farmed Atlantic salmon infected with *L. salmonis* (Huntsman 1918; White 1940, 1942; Brandal et al. 1976; Wootten et al. 1982; Jónsdóttir et al. 1992). The prevalence and severity of sea louse lesions in this study were greater than previously reported for sockeye. In this study the majority of fish had lesions that could be attributed to sea louse feeding. Abrasions and open wounds caused by sea lice were reported previously on the dorsal surface and in the perianal region of late-run sockeye in the Fraser River; however, data on lesion prevalence were not given (Gilhousen 1989). In contrast, Ishida and Nagasawa (1993) examined 2038 sockeye from the North Pacific Ocean, Bering Sea, and Ohkotsk Sea and found no lesions attributable to sea lice.

In 1990, low river flows and high river temperatures forced a high percentage (approximately 60%) of sockeye to remain in the inlet until September, thereby exposing the fish to an extended period of crowding, high water temperatures, and low dissolved oxygen levels (K.D. Hyatt, unpublished data). In our 1990 abundance samples, the highest proportion of adult *L. salmonis* present was ovigerous females. Each female carried high numbers of eggs (440 ± 120; mean ± SD) and was capable of extruding a second pair of egg strings within 2 days of the eggs hatching (S.C. Johnson, unpublished data). This strongly female sex ratio, high fecundity, and rapid development at higher temperatures (egg to infectious copepodid stage in 7.4 days at 15°C; Johnson and Albright 1991*b*) combined with the presence of a large number of hosts in a restricted area most likely allowed the population of *L. salmonis* to increase rapidly, resulting in the development of serious lesions while the fish were holding in the inlet. In 1992 and 1993, escapement to both Sproat and Great Central lakes followed a more normal pattern, with approximately 65% of the escapement entering the lakes before September 1 (K.D. Hyatt, unpublished data), and damage from sea lice was less severe.

In both the inlet and river samples of 1992 and 1993, the severity of sea louse lesions increased towards the end of the runs (September). A similar trend was reported by Gilhousen (1989), who found no sea louse lesions on Fraser River sockeye collected in July and August but abraded areas and open lesions on sockeye collected in September and October. It is possible that the longer period of residence in coastal waters allows a greater proportion of the copepods to develop to the preadult and adult stages. It is these stages that are most commonly responsible for disease because of their larger size and capability of moving on the host.

It is highly probable that sockeye, as they mature or are exposed to other stresses such as adverse environmental

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Table 4. Percentage of sockeye salmon (*Oncorhynchus nerka*) with open lesions (i.e., extending to the musculature) in the fall of 1990.

Date (in 1990)	Location	Sampling method	*n*	Unmarked	Mild external damage	Moderate external damage	Severe external damage
			Sproat Lake				
Sept. 14	Fishway	Trap	50	10	28	48	14
Oct. 26	Two Rivers Arm	Seine	100	88	9	2	1
Oct. 25	Taylor Arm	Seine	100	82	14	4	0
Nov. 5	Two Rivers Arm	Seine	100	94	4	2	0
Nov. 5	Taylor Arm	Seine	100	96	1	3	0
Nov. 19	Two Rivers Arm	Seine	100	100	0	0	0
Nov. 19	Taylor Arm	Seine	100	97	3	0	0
			Great Central Lake				
Sept. 14	Fishway	Trap	50	22	44	35	0
Sept. 25	Fishway	Trap	50	32	48	20	0
Oct. 26	Forestry Camp	Seine	82	84	12	4	0
Oct. 26	Fawn Point	Seine	100	82	9	7	2
Nov. 6	Forestry Camp	Seine	100	89	8	3	0
Nov. 7	Fawn Point	Seine	100	88	11	1	0
Nov. 26	Forestry Camp	Seine	100	83	12	5	0
Nov. 21	Fawn Point	Seine	100	85	13	2	0

Note: Fish were sampled at the fishways of the Sproat and Stamp rivers and various lake locations. Fish were ranked as having mild (one open lesion), moderate (two or three open lesions), or severe (more than three open lesions) external damage.

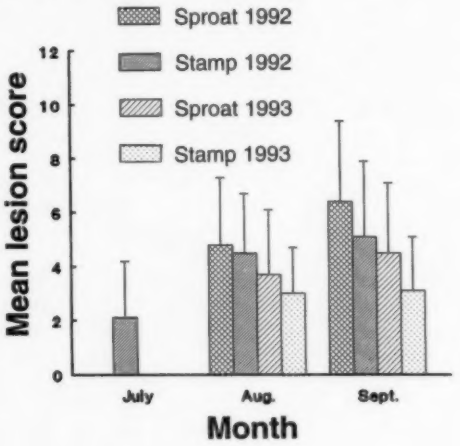
conditions (i.e., high temperature, low dissolved oxygen levels, or toxic plankton blooms), become more susceptible to infection with *L. salmonis*. Stress factors such as sexual maturation and exposure to deleterious environmental conditions are known to cause immunosuppression resulting in increased susceptibility of salmonid fishes to infection with a variety of parasites (reviewed in Pickering 1987). Immunosuppression of coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*), induced by implantation of hydrocortisol, increased their susceptibility to infection by *L. salmonis* (Johnson and Albright 1992; S.C. Johnson, unpublished observation).

Throughout the study, sockeye returning to Sproat Lake were more severely affected by sea lice than those returning to Great Central Lake. There are several reasons that may account for this difference. The longer migration into Great Central Lake may cause fish with more severe lesions to die before they reach the lake. This is unlikely, however, because (*i*) the passage into Sproat Lake is more difficult because of river topography and (*ii*) transit times into the two lakes do not differ substantially (Manzer et al. 1985). Furthermore, high numbers of heavily affected, moribund, or dead fish were not observed in either the Stamp, Sproat, or Somass rivers in 1992 or 1993.

Differences in patterns of migration on the high seas, timing of the return to coastal waters and the inlet, or other behaviors may expose Sproat or Great Central stocks to different levels of infection with *L. salmonis*. However, available data provide no consistent, demonstrably significant differences between these stocks with respect to migration patterns or timing of return to coastal waters and Alberni Inlet (K.D. Hyatt, unpublished data).

It is also possible that the two stocks differ in their susceptibility to infection with *L. salmonis* because of genetic differences. Differences in susceptibility to infection with parasites between stocks of coho and chinook salmon have been shown to have a genetic basis (Hemmingsen et al. 1986; Ching and Parker 1989). Great Central and Sproat stocks have been shown to consistently differ in both electrophoretic and DNA patterns (Quinn et al. 1987; Beacham et al. 1995; Taylor et al. 1996).

Fig. 4. Mean (+SD) monthly lesion scores for sockeye (*Oncorhynchus nerka*) from the Sproat and Stamp rivers collected in August and September of 1992 and 1993.



In 1990, samples collected on the spawning grounds of both lakes contained a higher percentage of fish without lesions than the river samples. These data suggest that lesions caused by sea lice may contribute to prespawning mortality during their stay in fresh water. Fish with open lesions would be more susceptible to secondary fungal and bacterial infections, as well as being osmotically compromised. In addition to direct mortality, there may be other subtle effects of sea louse damage on reproductive success. Berg et al. (1986) noted that wounded Atlantic salmon exhibited behavioral differences that decreased their reproductive success.

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Table 5. Percentage of sockeye salmon (*Oncorhynchus nerka*) from the Sproat and Stamp rivers with lesions attributed to the feeding activities of *Lepeophtheirus salmonis* by body region and river system in 1992 (July–September) and 1993 (August–September).

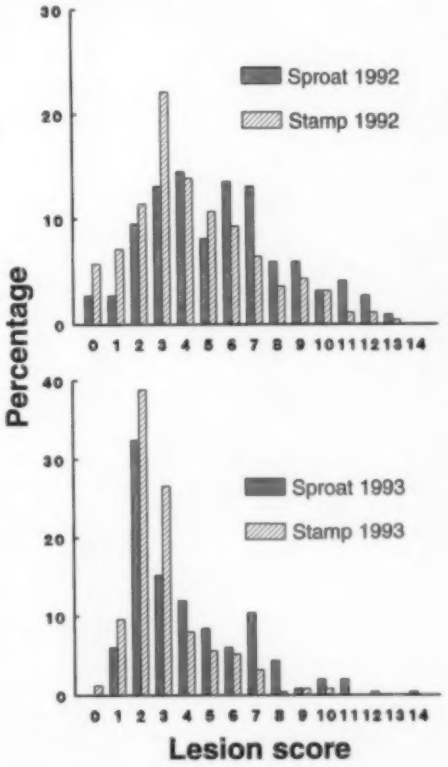
Lesion position, river system, and year	Lesion ranking						No. of fish examined
	0	1	2	3	4	5	
Posterior to dorsal fin							
Sproat River, 1992	5.5	34.1	20.0	10.0	8.6	21.8	220
Stamp River, 1992	13.3	33.7	28.3	10.4	3.9	10.4	279
Sproat River, 1993	9.6	54.4	7.2	6.8	2.8	19.2	250
Stamp River, 1993	14.0	68.4	7.2	2.8	3.2	4.4	250
Base of dorsal fin							
Sproat River, 1992	23.2	70.9	2.7	1.8	0	1.4	220
Stamp River, 1992	38.0	59.1	1.4	1.1	0	0.4	279
Sproat River, 1993	55.6	42.0	0.4	0	0.4	1.6	250
Stamp River, 1993	65.6	32.8	0.8	0.4	0	0.4	250
Head							
Sproat River, 1992	72.3	10.9	1.8	4.1	4.1	6.8	220
Stamp River, 1992	82.8	2.5	1.8	2.9	2.5	7.5	279
Sproat River, 1993	99.2	0	0	0.4	0	0.4	250
Stamp River, 1993	98.8	0	0	0.8	0	0.4	250
Perianal							
Sproat River, 1992	4.1	72.4	6.8	15.4	0.5	0.9	220
Stamp River, 1992	10.8	76.0	2.2	10.4	0	0.7	279
Sproat River, 1993	0	68.8	17.2	8.8	2.4	2.8	250
Stamp River, 1993	2.0	74.4	14.4	7.2	0.8	1.2	250

Because our samples were taken in September 1990, it is possible that we missed a large percentage of undamaged fish entering the lakes during earlier periods of escapement. However, a large percentage of the run would have had to occur prior to September to account for the observed low prevalence of open lesions on the spawning grounds. This was not the case in 1990, when approximately 69% of the run passed from Alberni Inlet into the Somass River after September 1 (K.D. Hyatt, unpublished data).

Nagasawa et al. (1993) observed slight increases in the prevalence, mean intensity, and abundance of *L. salmonis* on sockeye with increasing age and host size. However, sockeye in their study had very low intensities of infection (≤3 copepods/host) and did not have lesions induced by sea lice. Significant positive correlations between LS and hypural length and sea age were observed in both the Stamp and Sproat rivers in 1992; however, these were not repeated in 1993. The positive relationship demonstrated for 1992 is expected because fish with a longer sea residence would accumulate more sea lice and suffer more damage from their feeding activities. The lack of significant correlations in 1993 may be due to the broad categories used in our ranking system. Subtle differences in lesion severity within ranks, therefore, are not distinguished. In years such as 1993 when the majority of fish had only very mild lesions (rank 0 or 1), our ranking system may not be sensitive enough to identify correlations.

In summary, we saw higher prevalences and intensities of *L. salmonis* on mature sockeye in Alberni Inlet than reported for immature and maturing sockeye collected on the high seas. Sockeye may become infected with *L. salmonis* as they pass through coastal waters or hold in inlets prior to entering fresh water. It is also possible that immunosuppression concomitant with maturation makes sockeye more susceptible to infection with sea lice as they come ashore. Stocks of Alberni sockeye

Fig. 5. Distribution of lesion scores for sockeye (*Oncorhynchus nerka*) from the Sproat and Stamp rivers collected in August and September of 1992 and 1993.



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were shown to differ in the severity of lesions caused by sea lice. The reasons for this difference are unknown. In years, such as 1990, when unfavorable river conditions force the fish to remain in the inlet longer than normal, serious disease and mortality induced by sea lice can occur. In extreme cases, disease induced by sea lice appears to cause prespawning mortality in freshwater environments, thereby further affecting the reproductive success of these stocks. This observation highlights the important role that diseases may play in the population dynamics of fish stocks.

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PERSPECTIVE

Evaluation of the biological basis for a predator control program on northern squawfish (*Ptychocheilus oregonensis*) in the Columbia and Snake rivers

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Abstract: Over 900 000 northern squawfish (*Ptychocheilus oregonensis*) were removed from the Columbia and Snake rivers from 1990 through 1995 by sport-reward and directed fisheries in an attempt to increase production of salmon and steelhead (*Oncorhynchus* spp.). Our review suggests that low exploitation of northern squawfish can have disproportionately large benefits for salmon survival if exploitation does not stimulate a compensatory response among predator populations. The number of adult salmonids can be expected to increase with increased freshwater survival of juveniles at the prevailing depleted numbers. Northern squawfish consumed significant numbers of uninjured juveniles that would otherwise have survived migration. Northern squawfish consumed an estimated 16 million migrants per year or about 8% of the population. Population simulations suggest that the number of large piscivorous northern squawfish can be significantly reduced by exploitation rates of only 10–20%, which removal efforts from 1990 to 1995 have demonstrated are feasible. Compensation by northern squawfish or other predators is possible but is not guaranteed because of the relatively small fraction of the northern squawfish population that is affected by exploitation. We propose that evaluations of northern squawfish removal be based on indirect measures because direct demonstration of salmon survival benefits is not feasible.

Résumé : Plus de 900 000 sauvagesses du nord (*Ptychocheilus oregonensis*) ont été prélevées dans le Columbia et la rivière Snake, entre 1990 et 1995, par la pêche sportive avec primes et la pêche dirigée, dans l'espoir d'accroître la production de saumons et de truite arc-en-ciel anadrome (*Oncorhynchus* spp.). Notre étude indique qu'un faible prélèvement de sauvagesses du nord peut avoir des effets positifs, et hors de proportion, sur la survie des saumons si ce prélèvement ne suscite pas une réaction de compensation parmi les populations prédatrices. On peut prévoir une augmentation du nombre de salmonidés adultes si la survie des juvéniles en eau douce s'améliore, étant donné que les effectifs sont actuellement très affaiblis. Les sauvagesses du nord consomment des quantités importantes de juvéniles indemnes qui auraient, en leur absence, survécu à la migration. On estime à 16 millions par an, soit 8% de la population, le nombre de poissons en migration consommés par ce prédateur. Des simulations des populations indiquent que le nombre de sauvagesses piscivores de grande taille pourrait être réduit de façon notable par des taux d'exploitation de 10 à 20% seulement, résultat que les prélèvements réalisés de 1990 à 1995 permettent de considérer comme réalisable. Une réaction de compensation chez les sauvagesses ou d'autres prédateurs est possible mais non certaine, étant donné la fraction relativement petite de la population de sauvagesses du nord qui est touchée par l'exploitation. Nous proposons de fonder les évaluations du prélèvement de sauvagesses du nord sur des mesures indirectes car il n'est pas possible de démontrer directement son effet positif sur la survie des saumons.
[Traduit par la Rédaction]

Introduction

A large-scale project is currently removing northern squawfish (*Ptychocheilus oregonensis*) from the Columbia and Snake rivers in an attempt to reduce predation on juvenile anadromous salmonids during downstream migration. Predator control is a key element in programs to restore production and preserve endangered stocks of anadromous salmon and steelhead (*Oncorhynchus* spp.; National Marine Fisheries Service 1995; Northwest Power Planning Council 1995), which have declined concurrent with hydropower development in the Columbia River basin (Salo and Stober 1977; Chapman 1986; Nehlsen et al. 1991). Effects of predation and control of northern squawfish have been periodically considered for the Columbia River since at least 1953 (Zimmer 1953; Thompson 1959*a*, 1959*b*; Thompson and Morgan 1959; Washington Department of Fisheries 1962; Sims 1979; Mullan 1980; Uremovich et al. 1980; Rulifson 1984; Gray and Rondorf 1986). Formal northern squawfish removal efforts began in 1990 and

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Fig. 1. Main-stem dams of the lower Columbia and Snake rivers and the area included in the northern squawfish harvest program.

currently include 1038 km of river from the estuary upstream in the Columbia River to Priest Rapids Dam at river km 639 and upstream in the Snake River to Hells Canyon Dam at river km 399 (Fig. 1).

Benefits of the northern squawfish removal program are difficult to measure on the basis of time series or treatment-and-control comparisons of adult salmonid returns or juvenile salmonid survival. Adult returns are affected by many factors including other enhancement measures; hence, it is impossible to distinguish the relative effects of any single factor. Measurement of salmonid survival during migration through any given reach is also fraught with uncertainty, making it difficult to detect changes in reservoir mortality (Berggren and Filardo 1993; Smith et al. 1993), although advances in tagging, tag detection, and statistical estimators show promise for the future (Skalski and Giorgi 1993; Skalski et al. 1993). Evaluations based on adult returns or salmonid survival cannot be undertaken until enough northern squawfish are removed to have a significant effect and possibly until migrants return from 2 or 3 years in the ocean. In this case, failure to achieve goals might not be detected until after very large but ineffective expenditures were made on the program for an extended period.

Because of the difficulties of directly measuring benefits of northern squawfish removal, evaluations have been based on examination of hypotheses underlying the basic thesis that low exploitation of northern squawfish can have disproportionately large benefits for salmon. Underlying hypotheses include the following. (*i*) The number of returning adults is a function of survival of juvenile salmon and steelhead during migration in fresh water. (*ii*) Northern squawfish in main-stem Columbia and Snake River reservoirs consume significant numbers of juvenile salmon and steelhead that would otherwise have survived migration. (*iii*) Large, old northern squawfish are the most important predators of salmonids. (*iv*) The cumulative effect of a 10–20% annual exploitation rate reduces the predation rate from northern squawfish by 50% by reducing the population of older predaceous individuals. (*v*) Target exploitation rates can be achieved and sustained with a combination of removal methods. (*vi*) Northern squawfish population

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dynamics or the composition of the resident fish community do not compensate for removals.

In this paper, we review evidence for each hypothesis underlying the northern squawfish removal program, and we discuss prospects for achieving and demonstrating success.

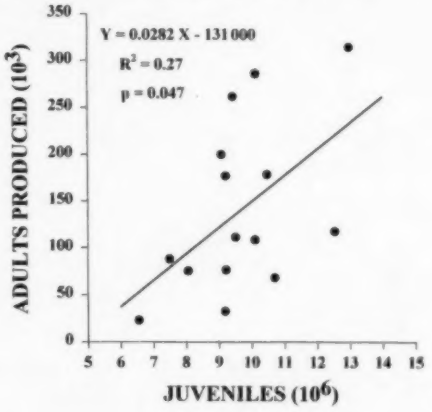
Relation of adult salmonid abundance to juvenile survival

Columbia basin salmonid stocks are predominately the following: spring-run chinook salmon (*Oncorhynchus tshawytscha*), which enter the river in March–May and spawn in headwaters during early fall; fall-run chinook salmon, which enter fresh water in August–September and spawn in river main stems during fall; and summer-run steelhead (*Oncorhynchus mykiss*), which return to fresh water in March–October and spawn in tributaries from January through June of the following year (Oregon Department of Fish and Wildlife and Washington Department of Fish and Wildlife 1995). Coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*Oncorhynchus nerka*), summer-run chinook salmon, and winter-run steelhead are also present but are most abundant in tributaries near the ocean or consist solely of small remnant populations (Oregon Department of Fish and Wildlife and Washington Department of Fish and Wildlife 1995). Juvenile salmonids typically migrate downstream through Columbia and Snake River reservoirs from April through August (Fish Passage Center 1994). Migrants during spring (April–June) are mostly yearling steelhead and spring-run chinook salmon with average fork lengths ranging from 165 to 225 and from 100 to 160 mm (Fish Passage Center 1994). Migrants during summer (July–August) are mostly subyearling fall-run chinook salmon, which average 100–115 mm in fork length (Fish Passage Center 1994).

A fundamental assumption of the Columbia River salmon restoration effort is that the number of adults recruited is a simple, positive response to the number of smolts produced (Whitney et al. 1993). Many of the measures taken or proposed to restore anadromous production have focused on improving survival of juvenile migrants severely affected by construction of a series of main-stem dams (Slatick et al. 1975; Ebel 1977, 1980; Raymond 1979, 1988; Wood 1993). Simulations with various life-cycle models indicate that mortality of juveniles during migration in fresh water constrains anadromous salmonid production in the Columbia River basin and reduces the benefits of enhancement measures upstream. These simulations generally assume that compensation in the ocean is weak.

The importance of compensation among salmon populations during ocean residence has been a subject of some debate within the fisheries community. Peterman (1980) concluded that there is reason to doubt the prevailing belief that marine survival of salmon is not density dependent on the basis of a review of juvenile and adult abundance data from a variety of North Pacific salmonid stocks, and he interpreted a low incidence of density dependence among stocks as the result of present abundances, which are too low to cause significant interaction. Nickelson (1986) concluded that coho salmon survival was density independent in the ocean off Oregon and that other analyses, which suggested density dependence for Oregon coho, were confounded by a change in stock composition from wild to hatchery fish. However, Peterman (1989) suggested that the low statistical power in Nickelson's failure to reject a null hypothesis of density independence introduced a high risk of mistakenly assuming density-independent marine survival of coho salmon.

Fig. 2. Regression of total number of adult spring chinook and steelhead returning to the Columbia River relative to estimated number of spring-migrating smolts passing the first dams they encountered on the mid-Columbia and Snake rivers. Data are from Raymond (1988) for 1970–1984 smolt migration years (all main-stem dams were operational beginning in 1970).



Data reported by Raymond (1988) confirm a significant ($p < 0.05$) linear relationship between spring chinook salmon and steelhead juveniles migrating during spring of 1970–1984 and the number of adults recruited for at least this portion of Columbia and Snake River salmonid populations (Fig. 2). Salmon returns to the Columbia River averaged 1.4 million adults from 1970 to 1994 (Oregon Department of Fish and Wildlife and Washington Department of Fish and Wildlife 1995), which is less than 20% of the 7.5–8.9 million adults produced annually before freshwater development (Chapman 1986). These results suggest that smolt and adult numbers are linearly related at current low run sizes and that density dependence may be expected only at much greater numbers.

Predation mortality

Research in 1983–1986 concluded that resident predator fishes consumed between 1.9 and 3.3 million juvenile salmon and steelhead (95% confidence interval) annually in John Day Reservoir alone (Rieman et al. 1991). Northern squawfish accounted for 78% of the losses, which would be 1.5–2.6 million fish or 7–17% of the run of 19 million salmon migrants. Individual consumption rates were low (reservoir-wide average <0.2 salmonids·predator^{-1}·day^{-1} based on stomach samples and digestion rate experiments; Vigg et al. 1991; Rieman et al. 1991), but the squawfish population was large (85 000 >250 mm fork length (FL) with 95% confidence intervals of –23 to 28% based on average for mark–recapture estimates in 3 years; Beamesderfer and Rieman 1991). Petersen (1994) determined that loss estimates to northern squawfish decreased to 1.4 million per year (7% of run) if stratified by four or more reservoir areas rather than the two areas used by Rieman et al. (1991).

Northern squawfish predation throughout the Columbia and

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Table 1. Projected abundance of northern squawfish, salmonid consumption rates, and losses of juvenile salmonids to predation by northern squawfish based on 1983–1986 mark–recapture estimates in John Day Reservoir (Beamesderfer and Rieman 1991), estimates of predation losses (Petersen 1994), live proportion (85%) from feeding experiments (Petersen et al. 1994), and index sampling throughout the Columbia and Snake rivers in 1990–1993 (Ward et al. 1995).

Location	Length (km)	Northern squawfish abundance (thousands)	Consumption rate (live smolts·predator^{-1}·day^{-1})	Losses (millions)
Estuary–Bonneville	224	734	0.09	9.7
Bonneville Reservoir	74	208	0.03	1.0
Dalles Reservoir	38	117	0.13	2.3
John Day Reservoir	123	85	0.09	1.2
McNary Reservoir	98	58	0.07	0.6
Priest Rapids Reservoir	29	28	0.05	0.2
Wanapum Reservoir	61	168	<0.01	0.2
Rock Island Reservoir	34	38	0.09	0.5
Rocky Reach Reservoir	68	142	<0.01	0.2
Wells Reservoir	47	39	0.01	<0.1
Ice Harbor Reservoir	51	14	0.02	<0.1
Lower Monumental Reservoir	46	53	0.01	0.1
Little Goose Reservoir	60	55	0.02	0.2
Lower Granite Reservoir	85	26	0.02	0.1
Total	1038	1765	0.06	16.4

Snake rivers was indexed in 1990–1993 on the basis of electrofishing catch rates of predators and the occurrence of salmonids in predator stomachs relative to estimates in John Day Reservoir (Ward et al. 1995). Northern squawfish abundance was estimated to total 1 765 000 and daily consumption rates averaged 0.06 salmonids/predator (Table 1). Average index values for predation losses relative to the estimate for John Day Reservoir were 808% downstream from Bonneville Dam, 275% in Bonneville plus The Dalles reservoirs, 50% in McNary Reservoir, 98% in Columbia River reservoirs upstream from the Snake River, and 37% in the four lower Snake River reservoirs (Table 1). These index values would translate into 16.4 million juvenile salmon and steelhead consumed annually by northern squawfish on the basis of the numbers observed in John Day Reservoir (Table 1) or 8% of the approximately 200 million hatchery and wild juvenile salmonid migrants in the system. Other work corroborates findings for the Snake River (Chandler 1993; Curet 1993) and the mid-Columbia between Priest Rapids and Chief Joseph dams (Burley and Poe 1994).

Although predation is intense near the dams, substantial predation also occurs outside the immediate vicinity of the dams. The boat-restricted zone of McNary Dam includes only 0.3% of the area in John Day Reservoir, but may contain 10% of the reservoir's northern squawfish population in some months; salmonid consumption rates were 3–14 times greater per predator (monthly averages) than in the rest of the reservoir, and net losses were more than 50 times greater than in the rest of the reservoir on a per unit area basis (Rieman et al. 1991). However, Petersen (1994) estimated that predation outside of the boat-restricted zone of McNary was 56–65% of the total loss and that 31–45% of the total loss was at least 10 km away. Ward et al. (1995) estimated that 48% of predation by northern squawfish in eight Columbia and Snake River reservoirs occurs within the reservoir rather than in the tailraces immediately downstream from dams.

Estimates of predation losses were relatively unbiased by consumption of salmonids killed or injured by dam passage. Passage mortality is a major source of freshwater mortality, which could confound estimates of predator effects on migrant survival if many of the salmonids eaten by predators were scavenged from those killed by dam turbines. However, most of the salmonids eaten by northern squawfish were eaten alive, despite observed preferences for dead salmonids in laboratory and field tests (Gadomski and Hall-Griswold 1992; Petersen et al. 1994). Petersen et al. (1994) marked and released dead and live salmonids into a dam tailrace in a 10% dead proportion that equaled the turbine mortality rate and observed that 22% of the marked salmonids subsequently recovered from northern squawfish stomachs were dead before release. If dead fish constitute 22% of northern squawfish prey near a dam, dam effects extend 10 km upstream and downstream, and 69% of predation occurs in that zone (Petersen 1994), then 85% of the estimated predation would be on live fish ($1 - 0.69 \times 0.22$).

Uncertainty in estimates of predation losses was estimated by Rieman et al. (1991) at ±25% (95% CI) on the basis of the inherent sampling variability in parameter estimates. Approximate 95% CIs (based on ±2SE) would be ±60% for restratified predation estimates by Petersen (1994). Assumptions about northern squawfish distribution, movements, and area-specific catchabilities could result in a fivefold underestimation of abundance of squawfish and losses of salmonids (Beamesderfer and Rieman 1988, 1991). Estimates of predation losses based on examination of stomach contents could also be conservative because squawfish kill prey that they do not ingest (Gadomski and Hall-Griswold 1992) and because gorging when prey are abundant may result in rapid pumping of undigested salmon through the alimentary tract (Thompson 1959*a*).

Large estimates of predation losses are consistent with previously unexplained mortality of juveniles during downstream migration (Rieman et al. 1991). Dams and reservoirs are generally believed to have increased the incidence of predation over historic levels (Poe et al. 1994). Brown and Moyle (1981)

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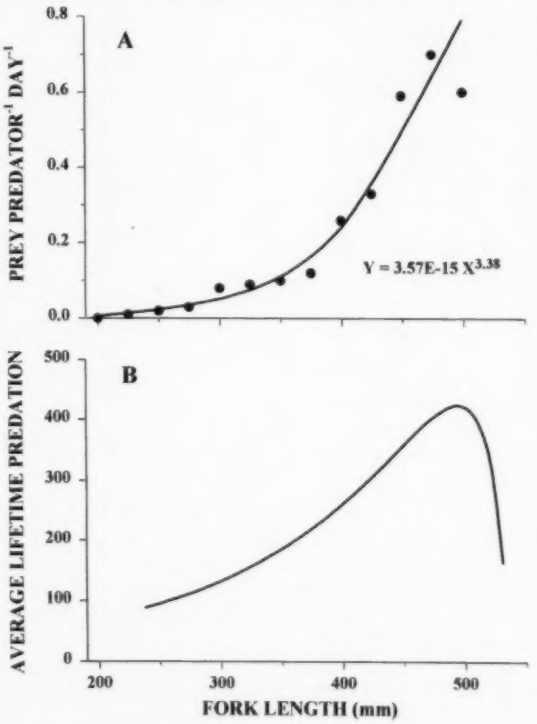
reviewed the literature on squawfish predation and concluded that lotic habitats and disruption of habitat or prey behavior may exacerbate squawfish predation. Impoundments in the Columbia River basin increase availability of microhabitats within the range preferred by northern squawfish (Faler et al. 1988; Beamesderfer 1992; Mesa and Olson 1993; Poe et al. 1994); increase water temperature, which increases digestion and consumption rates by squawfish (Falter 1969; Steigenberger and Larkin 1973; Beyer et al. 1988; Vigg and Burley 1991; Vigg et al. 1991); decrease turbidity, which may increase capture efficiency of predators (Gray and Rondorf 1986); favor introduced competitors, which could cause northern squawfish to shift to a diet composed largely of juvenile salmonids (Poe et al. 1994); and increase stress and subclinical disease of juvenile salmonids, which increases susceptibility to predation (Rieman et al. 1991; Gadomski et al. 1994; Mesa 1994). In addition, passage problems and reduced river discharge delay smolt migration, which increases exposure time to predation (Raymond 1968, 1969, 1979, 1988; Park 1969; Van Hyning 1973; Bentley and Raymond 1976) and also increases exposure later in the season when consumption rates are high (Beamesderfer et al. 1990; Rieman et al. 1991).

Northern squawfish life history

Northern squawfish are large, long-lived, slow-growing, predaceous minnows whose unexploited populations are typically dominated by large, old individuals. Maximum fork length, weight, and age are approximately 600 mm, 2.5 kg, and 16 years in the Columbia River (Rieman and Beamesderfer 1990; Parker et al. 1995). Annual mortality rates are 12–31% (Rieman and Beamesderfer 1990; Parker et al. 1995). Individuals 380 mm in length and greater constitute 12–59% of the population with FLs of 250 mm or greater (Parker et al. 1995). Sexual maturity occurs at sizes of 200–350 mm and ages of 3–8 years, with males typically maturing sooner than females (Beamesderfer 1992; Parker et al. 1995). Individual fecundity averages about 25 000 eggs/female (Parker et al. 1995). Spawning generally occurs in June and July in large aggregations that broadcast eggs over clean rocky substrate in slow-moving water at a wide range of depths in rivers, lake tributaries, lake outlet streams, and shallow and deep littoral areas (Beamesderfer 1992).

Diet of northern squawfish varies with size (Ricker 1941; Falter 1969; Olney 1975; Buchanan et al. 1981). In the Columbia River, invertebrates dominate the diet of fish smaller than 300 mm FL, with fish and crayfish increasing in importance as size increases (Thompson 1959*a*; Kirn et al. 1986; Poe et al. 1991, 1994). Salmonids, sculpins (*Cottus* spp.), trout perch (*Percopsis transmontana*), and suckers (*Catostomus* spp.) are the most common fish prey items of northern squawfish (Poe et al. 1991). Salmonids are generally an important diet component only for large, old northern squawfish (Vigg et al. 1991), and consumption rates of juvenile salmonids by northern squawfish increase exponentially as size increases (Fig. 3A). Consumption rates of juvenile salmonids also increase in areas of salmonid abundance (Thompson 1959*a*; Buchanan et al. 1981; Poe et al. 1991; Vigg et al. 1991; Tabor et al. 1993). Northern squawfish near the dams exhibit a classical functional response, where average predator consumption rates increase with increasing prey density up to a maximum corresponding to satiation (Henchman 1986; Vigg 1988; Petersen and DeAngelis 1992). However, the response appears to be weak in the reservoir body where most predation occurs (Fig. 4).

Fig. 3. Daily (A) and lifetime (B) consumption rates of juvenile salmonids for northern squawfish of various sizes based on natural mortality rates and diet analysis from John Day Reservoir in 1983–1986 (Rieman and Beamesderfer 1991; Vigg et al. 1991).



Exploitation effects

Simulations with a simple age-structured population model indicate that sustained annual exploitation of 10–20% of northern squawfish >250 mm FL will reduce predation by northern squawfish by 50% (Rieman and Beamesderfer 1990). This model, which is thoroughly documented in Rieman and Beamesderfer (1990), estimates predation losses as the product of age-specific predator number and size-specific predation rate in John Day Reservoir. Simulation results for John Day Reservoir will approximate the response in all areas because the relationship between consumption rate and size is similar. Age-specific number and size were calculated from annual mortality rate and a von Bertalanffy growth equation. Effects of density-dependent population regulating mechanisms were approximated by describing reproduction as Ricker or Beverton–Holt type functions of population egg production, which was based on size-specific female number and fecundity. Estimates of net predation were uniformly expressed as a percentage of the value for an unexploited population.

Rieman and Beamesderfer's (1990) simulations suggest

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Fig. 4. Relations between predator ration and abundance of juvenile salmonids near the upstream dam and in the remainder of the reservoir for John Day Reservoir, 1983–1986 (after Beamesderfer et al. 1990).

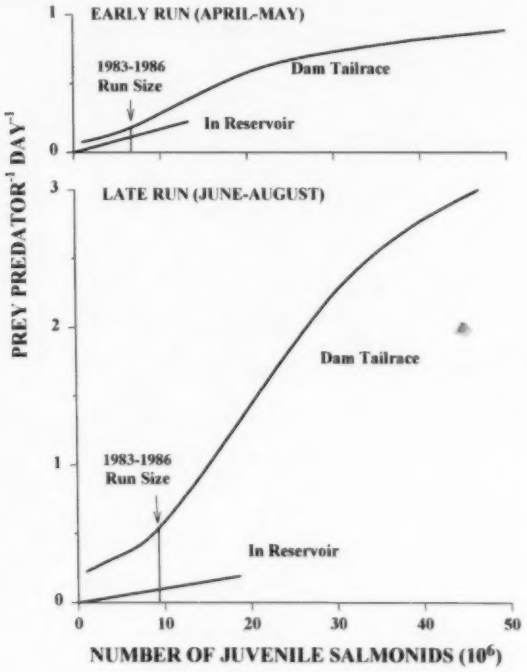
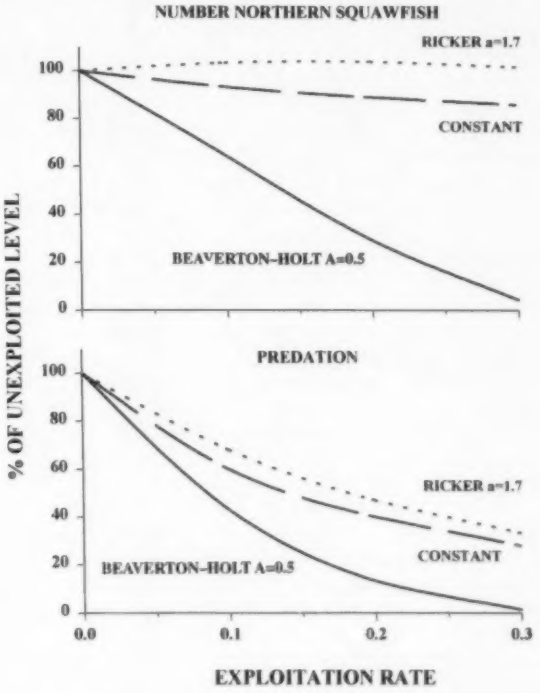


Fig. 5. Simulated response of northern squawfish abundance (all sizes) and predation to exploitation rate of northern squawfish >250 mm fork length based on data from John Day Reservoir in 1983–1986 (after Rieman and Beamesderfer 1990).



that a 50% reduction in predation requires a 10% annual exploitation rate (Fig. 5) if northern squawfish populations are relatively unaffected by density-dependent population-regulating mechanisms (i.e., where recruitment is approximately proportional to spawning stock size). A 20% annual exploitation rate is needed to reduce predation by 50% if compensatory regulating mechanisms are important (i.e., where recruitment is relatively constant over a wide range of spawning stock sizes). The primary control mechanism is the cumulative effect of exploitation, which systematically reduces the number of older piscivorous individuals through time. Effects of exploitation on spawner abundance and subsequent recruitment also contribute to reduced predation in simulations where density-dependent population-regulating mechanisms are unimportant.

Removals have little benefit during that same year because most salmonids pass before it is possible to catch significant numbers of predators (Beamesderfer et al. 1990). Instead, removal of a given fraction of the squawfish population reduces predation mortality in the following year. In general, an exploitation rate of 10% will reduce predation in the following year by 10% (Fig. 6). Equilibrium is reached in 10–15 years (corresponding to the difference between the age of recruitment to the removal program and the longevity of northern squawfish). Exploitation rates less than 10–20% also have significant effects. For instance, 5% exploitation will reduce predation by 15–30% at population equilibrium (Fig. 5). Greater exploitation rates produce a more rapid response (Fig. 6). For instance, a 20% exploitation rate will reduce predation by 50% relative the the original level within 5 years and by 57% at equilibrium.

Removal of each northern squawfish 250 mm in length or greater would save between 100 and 400 juvenile salmonids during the remainder of that predator's life, on the basis of the probability of survival to a given size and size-specific consumption rates in John Day Reservoir. Increases in average lifetime predation with increasing size up to 500 mm suggest that removal efforts targeting larger fish will be most effective. Removal of fish smaller than 250 mm would be expected to have little effect on predation because these fish do not prey on salmonids to any degree and their chance of surviving to predaceous sizes is remote.

Removal feasibility

Over 900 000 northern squawfish were removed from the Snake and Columbia rivers by a combination of methods during 1990–1995 (Table 2). Annual exploitation rates estimated at 9–16% on the basis of recaptures of tagged fish were generally within the target 10–20% range. Effective methods included paying recreational anglers $3 for each untagged fish (>280 mm FL) and $50 for each tagged fish returned to one of a series of check stations where the anglers registered each day prior to fishing. Recreational anglers averaged 62 000 trips/year and 2.5 fish/trip from 1991 to 1995 (Smith et al.

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1995). In addition, employees fished in selected sites of northern squawfish concentration near dams with hook and line and near hatchery release sites with gill nets (Collis et al. 1995, Columbia River Inter-tribal Fish Commission 1995). Employees fishing at hydropower facilities where public access was restricted averaged 12 700 h of effort/year and 1.7 northern squawfish/h from 1991 to 1993. Employees also averaged 4200 gill-net h/year and 4.9 fish/h from 1993 to 1995.

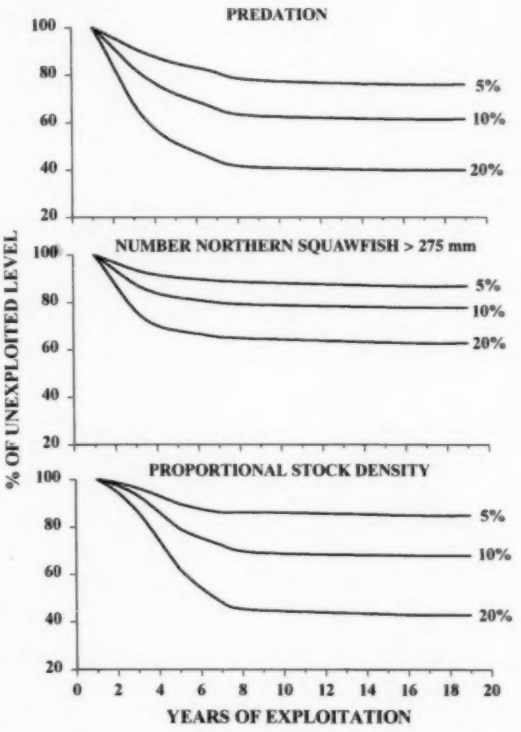
Intensive efforts to obtain exploitation rates greater than 9–16% have failed because of the large size of the system, low northern squawfish densities, and gear constraints of by-catch. The migration corridor in the Columbia and Snake rivers includes over 1000 km of river, an approximate surface area of 120 000 ha, and average depths of 7–20 m. Northern squawfish numbers systemwide are only 1700 fish/km or 15 fish/ha. An assortment of fishing gears has been deployed over the years with limited success, including gill nets (set or drifted, sinking or floating, horizontal or vertical), trap nets (Erie- and floating Merwin-style), seines (purse and beach), baited longlines, black cod traps (baited and unbaited), electrofishing boats, and even an electrofishing grid suspended from a dam. Several gears produced promising catch rates of northern squawfish during limited trials but failed during wider deployment to capture large numbers of northern squawfish without substantial by-catch of other species. For instance, by-catch of white sturgeon (*Acipenser transmontanus*) on baited longlines and of adult salmonids in floating Merwin traps far exceeded catch of northern squawfish when these gears were deployed by contract fishers from 1991 to 1993 (Mallette et al. 1994, 1995).

A large amount of energy and effort has also been spent attempting to locate northern squawfish spawning concentrations like those observed in other systems (Patten and Rodman 1969; Beamesderfer 1992). However, extensive sampling in tributaries and along shorelines of the Columbia and Snake rivers has failed to locate any significant concentration of spawners (U.S. Fish and Wildlife Service 1957; Reimers and Bond 1967; Ashe et al. 1995). Preferred spawning conditions of low water velocity and a clean rocky substrate (Beamesderfer 1992) are ubiquitous at all depths in the main-stem reservoirs. We conclude that northern squawfish are spawning in deep-water areas in each reservoir where current and structure prevent effective sampling.

Compensation potential

Benefits of northern squawfish exploitation could be less than expected if surviving northern squawfish or other predators increase their rates of predation, growth, or reproduction (Rieman and Beamesderfer 1990; Zimmerman and Parker 1995). However, salmonid predation is concentrated among large, old squawfish, which do not comprise a large fraction of the squawfish population. An exploitation rate of 10–20% on northern squawfish >250 mm in length has a small effect on the entire squawfish population. For instance, age-structured population simulations suggest that a 20% annual exploitation rate, which reduces predation by 63% upon equilibrium, will reduce the number of squawfish (all sizes) by only 12% upon equilibrium (Fig. 5). The population is restructured rather than significantly depleted and harvest may never reduce abundance enough to elicit a compensatory response.

Fig. 6. Estimated annual changes in potential predation and size structure of northern squawfish simulated at various exploitation rates for northern squawfish and constant number of recruits. Size structure is expressed as proportional stock density (PSD), which is the number of northern squawfish of fork length 380 mm and greater divided by the number longer than 250 mm and expressed as a percentage.



Evidence for or against compensation by northern squawfish or other predators is equivocal. The functional response suggests that feeding by remaining predators may increase with increased smolt survival following removal of predators upstream (Beamesderfer et al. 1990). However, there is only a small increase in the number of captures of prey per predator when the number of smolts increases in the reservoir where much of the predation occurs. A negative correlation has been identified between walleye (*Stizostedion vitreum*) and northern squawfish year-class strength (Connolly and Rieman 1988), but it is unclear whether this correlation results from an interspecific interaction or covariation with environmental conditions. No significant negative correlations were noted between northern squawfish density and growth, condition, fecundity, or annual mortality rate among nine populations examined by Parker et al. (1995), but all populations were unexploited.

Discussion

Predator control programs have a long history in natural resource management but have been widely discredited in the biological community. Many predator control programs failed

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Table 2. Number of northern squawfish >250 mm fork length removed from the lower Columbia River basin by fisheries and for evaluation, 1990–1995.

Year	Reward fishery	Dam angling	Site-selective fishery	Evaluation sampling	Exploitation rate
1990	4 681	11 005	1420	3292	
1991	153 508	39 351	1003	1853	11 (—)
1992	186 095	27 868	1535	2049	12.0 (8.0–15.9)
1993	104 536	17 210	1772	1758	8.6 (7.3–9.9)
1994	129 384	16 097	9017	1169	13.1 (11.7–14.6)
1995	199 600	5 488	9634	1396	15.6 (13.4–17.6)

Note: Confidence intervals (95%) for exploitation rate estimates (given in parentheses) are based on the proportion of marked fish recaptured in fisheries.

because prey populations were not limited by predation, success relied on removal of a large fraction of the predator population, control efforts could not be sustained as predator numbers declined, large removals stimulated a compensatory response in the predator population, or evidence for benefits was circumstantial (Hubbs 1940; Jacobsen 1945; Meachum and Clark 1979; Otis 1988; European Inland Fisheries Advisory Commission 1991; Goeman and Spencer 1992).

Control of predaceous fishes has proven successful in a few cases but examples are scarce. The success of lamprey (*Petromyzon marinus*) control in the Great Lakes is well documented (Smith and Tibbles 1980; Koonce et al. 1993). Control of Arctic char (*Salvelinus alpinus*) reduced predation on sockeye salmon during a 1-year experiment in Alaska's Wood River system (Meachum and Clark 1979). A long-term shark (*Carcharinus*, *Carcharadon*, *Galeocerdo*, and *Sphyrna* spp.) harvest program in Australia has reduced the number of dangerous sharks (Paterson 1990). A northern squawfish eradication program in Cultus Lake, British Columbia, was correlated with a substantial increase in the production of sockeye salmon (Foerster and Ricker 1941), although the mechanism for this response was unclear (Brown and Moyle 1981). Several experimental eradication programs targeted northern squawfish but were not continued as part of routine management for unknown reasons (Foerster and Ricker 1941; Jeppson 1957; Jeppson and Platts 1959; MacPhee 1969; Hamilton et al. 1970).

On the basis of our review of the evidence for underlying hypotheses, we conclude that a low rate of exploitation of northern squawfish can benefit salmon survival in the Columbia River if exploitation does not stimulate a compensatory response among predator populations. The number of adult salmonids can be expected to increase with increased freshwater survival of juveniles at the prevailing depleted numbers. Northern squawfish consume a significant fraction of salmonid juveniles, which would otherwise have survived migration. The number of large piscivorous northern squawfish can be significantly reduced by exploitation rates of only 10–20%. Finally, removal efforts from 1990 to 1995 have demonstrated that target exploitation rates can be achieved and sustained at least over a short term. The likelihood of compensation by northern squawfish and other predators remains unknown: compensation is possible but is not guaranteed because of the relatively small fraction of the northern squawfish population that is affected by exploitation.

Whitney et al. (1993) note that while few would argue that predation is not a source of mortality, a definite relationship between salmonid productivity and mortality of smolts owing to riverine predators has yet to be substantiated. We conclude that direct demonstration of the benefits of the northern squawfish removal program is not presently feasible and that evaluations must continue to rely on indirect measures. For instance, mark–recapture estimates of exploitation rate will determine if target exploitation rates are sustained. The relative abundance and size composition of northern squawfish populations will demonstrate whether the northern squawfish population responds as predicted. Similarly, time series of estimates of diet, growth rate, fecundity, and age at maturity of northern squawfish and relative abundance of other fish species may identify compensation by individual northern squawfish, the northern squawfish population, or the resident fish community that could negate the benefits of removal. Hilborn and Winton (1993) suggested that effective evaluation of enhancement programs may require 15 or more years of data. We recommend that evaluation of the predator removal program be based on a sustained annual sampling program to maximize the power of tests for a response and to factor out confounding effects of environmental variability.

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B.E. Rieman first recognized the feasibility of using small harvest rates of northern squawfish to produce large improvements in salmonid survival, and S. Smith predicted the potential of recreational anglers for achieving significant harvest. B.L. Uremovich and G.A. Gray helped rejuvenate predation research with their respective observations at Bonneville Dam and McNary Reservoir. W. Nelson, W. Maslen, and S. Vigg played crucial roles in program development. Constructive comments on earlier drafts of the manuscript were provided by M. Chadwick, J.H. Petersen, C.F. Willis, and two anonymous reviewers. This work was supported with funds from the Bonneville Power Administration.

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DISCUSSION

Comment: Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada[1]

A.F. Sinclair and D.P. Swain

Large differences in size at age and growth among cod stocks are well known. In waters off eastern Canada, size at age is smallest in the southern Gulf of St. Lawrence, intermediate on the Scotian Shelf (increasing from east to west), and largest in the Gulf of Maine. There was a large decline in cod size at age from the late 1970s to the mid-1980s in the southern Gulf of St. Lawrence and eastern Scotian Shelf that significantly reduced the productivity of these stocks. However, this did not occur in the western Scotian Shelf or Gulf of Maine areas. Campana et al. (1995), in an analysis of Canadian research vessel survey (RV) data on cod size at age and near-bottom temperatures in these areas, concluded that "the large-scale gradient in cod size at age . . . appears to be largely explicable on the basis of temperature alone" and that "long-term shifts in water temperature appear almost certain to produce concomitant shifts in the growth rate of cod." While there is a clear spatial correlation between cod size at age and temperature in this area, their analysis does not reveal a convincing causal relationship between the two.

No attempt was made to test the importance of alternative mechanisms controlling size at age, a necessary step before concluding that temperature alone is largely causing the observed variation. Other factors that are known to affect fish growth, such as density, seasonal migrations, and genetics, also vary across the study area and among these cod stocks. Cod densities show the opposite gradient to temperature and this too may explain the differences in observed patterns in size at age through density-dependent effects. The 1983–1994 average age-5 RV mean catches per tow for the southern Gulf of St. Lawrence, eastern Scotian Shelf, and western Scotian Shelf stocks were 21.1, 7.8, and 2.0 kg/tow, respectively (calculated from data in Sinclair et al. 1995, Fanning et al. 1995, and Clark et al. 1995). Southern Gulf of St. Lawrence cod, the smallest cod in the study, undergo extensive seasonal migrations, making an approximately 1000-km round trip, leaving the Gulf in the fall to spend the winter in the Laurentian Channel, then returning in spring to spawn. Presumably this would use energy that could otherwise be directed towards growth. Seasonal migrations in the other areas are much less extensive. Genetics-based stock identification projects are currently underway in the study area; can genetic differences between populations influence size at age?

The statistical analysis suggests that stock attributes, which may reflect some of the factors mentioned above, have a greater effect on size at age of cod than temperature alone. The addition of temperature to a model with age and stock was not significant ($p = 0.110$) whereas the addition of stock to a model with age and temperature was ($p = 0.006$) (their Table 2). Lack of fit to the age–temperature model is indicated by the spatial distribution of residuals in their Fig. 9. Temperature alone was a poor predictor of size at age on the western Scotian Shelf and Bay of Fundy where uniformly large and positive residuals were found. Residuals were mainly negative in the eastern portion of the eastern Scotian Shelf (4Vs and 4Vn) and mainly positive on the central Scotian Shelf (4W). Within the southern Gulf of St. Lawrence, residuals were mainly negative in the western area and positive in the eastern area. Thus, our qualitative interpretation of this residual pattern is different from the authors'. We find it to be strongly associated with cod populations, and are not surprised that stock is a better predictor of size at age than temperature alone.

Temperature at capture is used to predict cod size at age. However, temperature varies from year to year, as does cod distribution with respect to temperature (Page et al. 1994; Swain and Kramer 1995). Temperature at capture provides no information on the annual fluctuations that may be related to annual variation in growth. Instead, a correlation between temperature at capture and size at capture must reflect persistent spatial variation in temperature and size and cannot be used to predict annual variation in size at age in a particular region or stock.

Campana et al. (1995) recognized the limitations of their model for testing effects of temperature on annual variations

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[1] Paper by S.E. Campana, R.K. Mohn, S.J. Smith, and G.A. Chouinard. 1995. Can. J. Fish. Aquat. Sci. **52**: 2445–2456.

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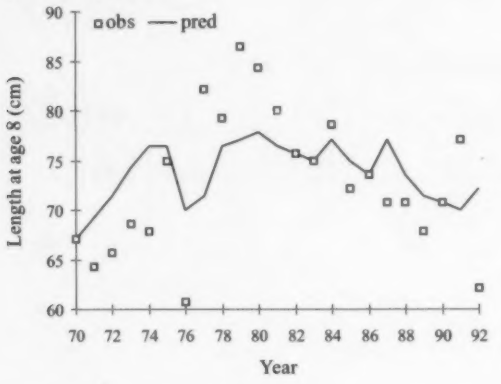
Fig. 1. Observed and predicted lengths of age-8 cod (cm) on the eastern Scotian Shelf between 1971 and 1992. Redrawn from Campana et al. (1995).



in size at age. Rather than using their model to directly predict the lengths of age-8 cod on the eastern Scotian Shelf in each year from 1970 to 1992, they claimed to have used observed initial length at age 2 and annual growth increments predicted from observed bottom water temperatures and their parameterized growth model. However, even using an increment approach, predicted age-8 length in a particular year will still depend only on temperature in that year, and not on the annual temperature history of the cohort. Length increments would be calculated as follows:

$$I_{3,j} = \hat{L}_{3,j} - L_{2,j-1}$$

where $I_{3,j}$ is the growth increment in year j for the cohort with age 3 in that year, $\hat{L}_{3,j}$ is the length at age 3 predicted from their model given the bottom temperature observed in year j, and $L_{2,j-1}$ is the observed age-2 length in year $j-1$. For older ages,

$$I_{i,j} = \hat{L}_{i,j} - \hat{L}_{i-1,j-1}$$

where i indexes age from 4 to 8. Then, length at age 8 in year j, predicted from annual growth increments, is given by

$$\begin{aligned}\hat{L}_{8,j} &= L_{2,j-6} + I_{3,j-5} + I_{4,j-4} + I_{5,j-3} + I_{6,j-2} + I_{7,j-1} + I_{8,j} \\ &= L_{2,j-6} + (\hat{L}_{3,j-5} - L_{2,j-6}) + (\hat{L}_{4,j-4} - \hat{L}_{3,j-5}) + \ldots \\ &\quad + (\hat{L}_{8,j} - \hat{L}_{7,j-1}) \\ &= \hat{L}_{8,j}\end{aligned}$$

That is, all of the terms based on earlier temperature conditions cancel, so that predicted age-8 length in year j depends only on temperature in year j.

Even if there are merits in how the lengths at age 8 were predicted from temperature data, we see little evidence that changes in temperature affected growth on the eastern Scotian Shelf. Their conclusion is based on a qualitative examination of their Fig. 11, which we have redrawn here changing the emphasis on observed and predicted values (Fig. 1). The authors state that the predicted lengths at age 8 reflected those observed, in particular with respect to the increase in lengths before 1980 and the subsequent decline. Our interpretation of the same figure is that the trend in predicted and observed values coincided somewhat in the early 1970s (though the scale was different) but that there was little to no similarity in the remaining period. The observed length at age 8 declined by about 15 cm from 1979 to 1987 whereas the predicted length from the temperature model showed no trend. In fact, there is no evidence that bottom temperatures on the eastern Scotian Shelf declined during this period, either in their Fig. 10 or in the environmental overviews reported by Drinkwater et al. (1995) and Page et al. (1995). The "cold" period on the eastern Scotian Shelf occurred in the late 1980s and early 1990s, well after cod lengths at age declined. Thus, we see no support here for the authors' contention that shifts in water temperature produced shifts in growth rates, either in general or on the eastern Scotian Shelf.

We question the exclusion of stations at depths of less than 50 m from the statistical analysis. The authors' rationale was that these depths were associated with warm, stratified sites with high seasonal variability in temperature. Stratification in these shallow waters is, in fact, weak. But more importantly, migratory stocks could experience a wide range of temperatures just by moving. Low capture temperatures of 0°C or less at depths of 60–100 m in the southern Gulf of St. Lawrence would be no more representative of annual temperature exposure than the higher temperatures at shallower depths. This dubious rule eliminated about one third of southern Gulf of St. Lawrence survey sets, almost half of the cod catch, and virtually all of the warm area for a stock with the lowest size at age. We see no compelling reason why so much data should be ignored. At the very least the authors should indicate the effect this had on their statistical analysis.

Campana et al. (1995) stated that a close agreement between predictions based on their model and observed lengths at age of other cod stocks supported their temperature-based growth model. We had difficulty reproducing the cited results in many cases. However, in cases where we were able to find the required data in the cited papers, we found that agreement between predicted and observed values was often poor for ages not cited by Campana et al. (1995). They indicated that age-6 predictions for Icelandic cod were within 7% of observed values based on data in Jonsson (1965). The same paper also presents data for ages 3–5, and the agreement is poorer for these ages, being within 27%, 17%, and 13% for ages 3–5, respectively. This level of agreement is poor, in our opinion, given that all predicted lengths were shorter than the observed, and that these prediction errors are as great as or greater than the observed annual growth increments. They cited comparisons for Barents Sea cod using February lengths at ages 4–6 from Nakken and Raknes (1987) and August bottom temperatures from Loeng (1990). This questionable match of temperature and size at age may partially explain why all predicted lengths, but one, were longer than the observed values. Data not mentioned by Campana et al. (1995) were also available for ages 3 and 7 and indicated prediction errors of 17% and 6%, respectively. We could not find the cited temperature data for west Greenland cod (July bottom water temperatures) in Elizarov (1965) and while Campana et al. (1995) cited calculations for age-5 cod only, there are data for ages 5–9 in Hermann and Hansen (1965). The only temperature data available for 3K cod in May et al. (1965) are in contour plots of temperature and depth. How was a single temperature measure obtained from these? Furthermore, Campana et al. (1995) chose not to show data for other areas and ages that were available in the same paper. We were unable to calculate

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predicted values in this case because we could not reproduce the temperature calculations.

It is likely that complex relationships determine cod growth and size at age in the wild. There is no doubt that temperature can influence fish growth, as can density, competition, genetics, and fishing. It is likely that no one factor can adequately describe the changes in cod growth that have been observed in these wild populations, and it may be that a combination of factors is at work. Elucidating causal factors and ranking their importance will be difficult given the problems of sampling, estimation, and experimentation. Comparative studies such as this one hold promise especially given the contrasts in growth rates, changes in growth rates, and trends in environmental conditions among areas. To be effective, however, the studies need to go beyond simply describing spatial patterns as Campana et al. (1995) have done, and investigate alternative mechanisms.

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Reply: Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada[1]

S.E. Campana, R.K. Mohn, S.J. Smith, and G.A. Chouinard

Sinclair and Swain (1996) have discussed in considerable detail their belief that temperature is of only modest consequence to the growth of cod, and that alternative factors are more influential in explaining the spatial and temporal trends that have been observed on the eastern coast of Canada. We welcome their interest in this subject, and fully support their plea for further research on the factors influencing growth rate. As was stated on numerous occasions in our paper, temperature alone cannot explain the observed growth patterns; other variables are almost certainly at play. Nevertheless, we were surprised and concerned by the emphasis that Sinclair and Swain placed on these possible alternatives, and by the extent to which they downplayed the influence of temperature. Indeed, in one of their opening comments, they stated that "while there is a clear spatial correlation between cod size at age and temperature in this area, their analysis does not reveal a convincing causal relationship between the two." Presumably, Sinclair and Swain did not intend to suggest that there is no causal relationship between temperature and growth rate in fish. Indeed, we believed that all those familiar with the subject of fish growth would accept the causal link as a given. In a review of the field of fish growth, Brett (1979) referred to temperature as the "controlling factor" of fish growth, through its direct effect on metabolic rate, oxygen consumption, feeding rate, digestive rate, activity, and scope for growth. He went on to indicate that the effects of temperature, first and foremost, must be accounted for before the effects of other "limiting" factors can be quantified. These sentiments were later echoed by Ricker (1979), and supported by the 2719 other papers (number based on a computerized literature search) published since 1978 that document a relationship between temperature and fish growth. Accordingly, we must disagree with Sinclair and Swain on the emphasis that should be placed on further research. The question is not whether temperature has a major influence on cod growth in space and time; the question is how large will its effect be?

To determine the expected magnitude of the temperature effect on cod growth, we must turn to the literature reporting the results of controlled experiments. There are surprisingly few papers that quantify and compare cod growth under controlled conditions of temperature, and most of those deal with juveniles. Nevertheless, the different sets of results are very consistent. Brown et al. (1989) reported a 40% increase in growth rate at length for cod reared at 8.3°C compared with 4.5°C. This value was similar to that of Otterlei et al. (1994), who reported a growth rate increase of about 50% with each 4°C increase in temperature between 6 and 14°C, and that of Waiwood (1978), who reported 33–50% higher growth rates for cod reared at 8°C compared with 4°C. Compare these experimental results with the 32% increase predicted by our growth model over the same temperature range (4–8°C). Clearly, our predicted effect of temperature is, if anything, somewhat smaller than that which is observed experimentally. While we do not suggest that temperature accounts for all of the observed growth rate variation in cod, both the experimental results and basic physiological theory indicate that it could (and should) account for a major proportion of it.

In attempting to build a case for alternative modifiers of cod growth, Sinclair and Swain questioned the accuracy and care with which our growth model was developed. These arguments were not well founded. In particular, their reconstruction of our predictive growth model for eastern Scotian Shelf cod was in error and was not that used or described in our paper. Their error appears to stem from their definition of $I_{i,j}$, which is not the same as that in Campana et al. (1995). The annual growth increment (I) along a cohort in year j was calculated in Campana et al. (1995) as

$$I_j = a + b \times \text{temperature}_j$$

where a and b are the age and (age × temperature) coefficients presented in the lower panel of Table 1 in Campana et al. (1995). Length at age i was then calculated as

$$L_{i,j} = L_{2,j-6} + \sum_{j-3}^{j} I_j$$

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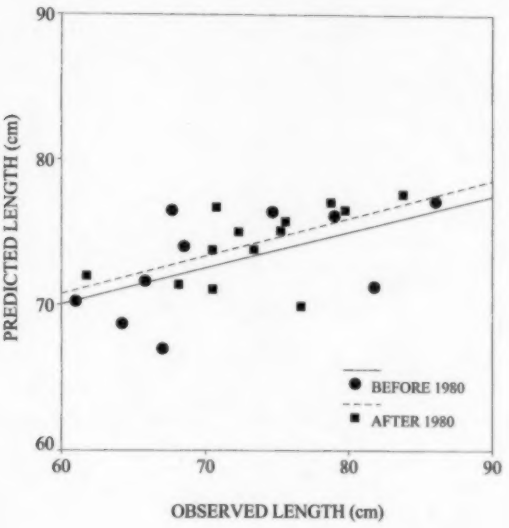
Fig. 1. Relationship between observed and predicted lengths of age-8 cod (centimetres) on the eastern Scotian Shelf between 1971 and 1992. The slope of the regression is statistically significant ($p < 0.05$) both for the whole time series and for the period from 1980 onwards.



Therefore, for an 8-year-old cod in year j, the calculation would be

$$\begin{aligned} L_{8,j} &= L_{2,j-6} + I_{j-5} + I_{j-4} + I_{j-3} + I_{j-2} + I_{j-1} + I_j \\ &= L_{2,j-6} + (a + b \times \text{temp}_{j-5}) + (a + b \times \text{temp}_{j-4}) \\ &\quad + (a + b \times \text{temp}_{j-3}) + \ldots + (a + b \times \text{temp}_j) \\ &= L_{2,j-6} + 6a + b(\text{temp}_{j-5} + \text{temp}_{j-4} + \ldots + \text{temp}_j) \end{aligned}$$

Contrary to the assertion of Sinclair and Swain, there is no cancellation of annual temperature terms in this calculation, and no undue effect of the temperature in the year of collection compared with earlier years.

Sinclair and Swain then asserted that there was no correlation between the growth model's predictions of length at age 8 and those that were actually observed in eastern Scotian Shelf cod. This statement is incorrect. Predicted and observed lengths at age 8 were significantly correlated ($p < 0.05$) throughout the time series (Fig. 1); they were also significantly correlated ($p < 0.05$) for the period from 1980 onwards, which is the period to which they pointed as showing no correlation (Fig. 1). Given the fact that temperature in this area has declined significantly ($p < 0.05$) since the mid-1980s (Page et al. 1994; Fig. 10 of Campana et al. 1995), and in light of the accepted causal relationship between growth rate and temperature, we infer that falling temperature contributed to the decline in growth rates. We agree with Sinclair and Swain that temperature alone cannot explain all of this decline, a point that we made in our original paper. However, we are at a loss to suggest an additional cause. While Sinclair and Swain suggested that density-dependent effects might be responsible, such effects were examined and later rejected by the stock assessment working group responsible for the area, owing largely to the fact that both growth rates and stock biomass have declined in concert in the past 10 years (Department of Fisheries and Oceans 1996).

Sinclair and Swain reported that they had difficulty reproducing our growth calculations for European cod stocks, and in some cases, were unable to locate the cited references. We are at a loss to explain their difficulty, as we have rechecked the accuracy of both our calculations and our references. All were correct as originally presented. Perhaps their problem stemmed from the error in their growth increment calculations noted above. As for their statement that we selected for presentation only those ages that matched our model predictions, we would be pleased to provide the growth comparisons for all age groups in each stock to any interested reader: such an examination would show that in each of the stocks we selected for presentation the oldest available age group was well represented within the age–frequency distribution.

We agree with Sinclair and Swain that what were termed "stock" effects in Table 2 of Campana et al. (1995) contributed significantly to the growth model. However, ambiguous terminology on our part has confused the issue. While they mistakenly attributed the term stock effects to genetic differences in growth rate, the intended meaning of the term was stock area effects, defined as the difference in growing days (loosely defined here as the number of degree-days over the temperature range in which the stock can be found) among stock areas. Such differences can be very large. For example, Swain and Kramer (1995) reported that 95% of the Gulf of St. Lawrence cod stock was found in temperatures of –0.25 to 9.7°C, a range that excludes temperatures present in the surface waters of the Gulf for 6 of the 12 months (Petrie 1990). On the other hand, 90% of the cod in the Bay of Fundy were found at temperatures between 4 and 12°C (Page et al. 1994), a range that excludes only one monthly average temperature in the environment (Petrie et al. 1996). Because temperatures tend to be highest during the summer (when the temperatures for the growth model were collected), and because the Bay of Fundy has the highest year-round temperatures, it follows that the research vessel temperature index would underestimate the growing period in the Bay of Fundy relative to the Gulf and other areas. Thus, it is not at all surprising that the model residuals were positive in the Bay of Fundy, and it is completely unnecessary to invoke hypothetical genetic differences. We would also note the results of two studies reporting the results of rearing genetically and environmentally distinct cod stocks together under identical environmental conditions. In both instances, the differences in growth rate were significant but small (<10%) (Naevdal et al. 1992; Svasand et al. 1996). Genetically induced differences in growth rate would have to be much larger (~300%) to be able to explain the observed growth rate variation in Scotian Shelf cod stocks.

Sinclair and Swain were critical of our decision to exclude stations at depths of less than 50 m in the Gulf of St. Lawrence, but do not appear to have considered the consequences of their proposal. The following data were drawn from Petrie (1990) and Petrie et al. (1996): at depths of 0, 50, and 100 m, summer temperatures were 13.0, 0.6, and 0.7°C, respectively, in the southern Gulf, and 10.4, 8.0, and 7.3°C, respectively, in the Bay of Fundy. Because the median temperature of association for cod in the Gulf in summer is 0.85°C (Swain and Kramer 1995), it is clear that surface water temperatures of 13°C are not an appropriate index of living temperature for southern

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Gulf cod. It is for that reason that Gulf cod captured at such shallow depths were not included in the growth model, despite the fact that their length at age was not significantly different from that of cod captured below 50 m. On the other hand, temperatures throughout the water column were much more similar in the Bay of Fundy, reflecting the fact that the waters in that area were much less stratified, and suitable for growth all year round. Thus we felt, and still feel, that elimination of depths of less than 50 m from the entire Scotian Shelf would have very little impact on most areas, but their exclusion makes the temperature index for the southern Gulf far more realistic.

Despite our disagreement with many of the points raised by Sinclair and Swain, we continue to support the general premise that factors other than temperature must be contributing to the observed spatial and temporal patterns in cod size at age. Yet the search for these alternative factors has, to this point, been disappointing. Sinclair and Swain suggested three potentially important modifiers of cod growth: density dependence, genetics, and fishing. Of these, evidence of density-dependent growth has been noted only in the southern Gulf of St. Lawrence cod stock, but even there, growth rates have remained low despite very low stock abundance (Sinclair et al. 1996). There has been no inverse relationship reported between growth rate and stock biomass in the other cod stocks of the Scotian Shelf; indeed, the opposite correlation is now present on the eastern Scotian Shelf. As for genetic effects, the two studies on cod reported only small (<10%) growth differences between genetically distinct stocks reared together under identical environmental conditions (Naevdal et al. 1992; Svasand et al. 1996). The final possibility, fishing effects, is much more difficult to evaluate, as it involves long-term genetic selection. However, it seems reasonable to acknowledge its existence, even if we are not yet able to quantify its impact.

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